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ORIGINAL ARTICLE

A Study on the Quality Evaluation of Agarwood of *Aquilaria sinensis* and *Aquilaria malaccensis* Induced by Different Inducers Based on Gray Correlation Degree and TOPSIS

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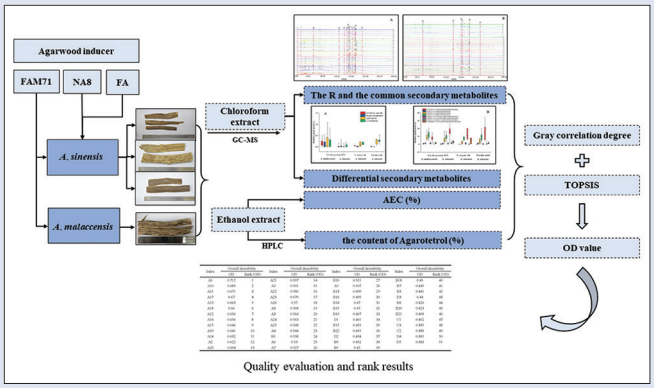
ABSTRACT

Background: Agarwood is a resinous heartwood produced in certain *Aquilaria* species that is often used as a spice and Chinese medicine materials. **Objectives:** This study aimed to comprehensively evaluate the agarwood quality of different species under the same inducer as well as different inducers of the same species. **Materials and Methods:** The GC-MS data of agarwood were retrieved by AMDIS to obtain 47 secondary metabolites. Combined with multivariate statistical analysis, 12 secondary metabolites were identified as potentially representing the differences between *A. malaccensis* and *A. sinensis*. The gray correlation degree and TOPSIS method were used to comprehensively grade 18 characters, including the AEC, the content of agarotetrol, the apparent abundance of GC-MS fingerprint, and the 15 secondary metabolites representing the 58 batches of samples. The OD values representing the overall desirability of r_i (gray correlation degree) and c_i (TOPSIS) were ranked, with a higher ranking reflecting, better agarwood quality. **Results:** The ranking results demonstrated that the agarwood samples of the top 33 in OD value were all induced by FAM71, whereas the agarwood samples of the top 23 were all from *A. malaccensis*. The agarwood of *A. sinensis* induced by FA had the lowest OD value. **Conclusion:** The study demonstrated that the quality of agarwood from *A. malaccensis* was better than that of *A. sinensis* using the same inducer of FAM71. In the same species of *A. sinensis*, the quality of agarwood produced by FAM71 was better than that induced by formic acid alone or NA8 alone. This study provided a theoretical basis for the selection of high-quality agarwood inducer and tree species, as well as a reference basis for the efficient production of agarwood in the actual production process.

Key words: Agarwood, agarwood inducer, *Aquilaria malaccensis*, *Aquilaria sinensis*, quality evaluation

SUMMARY

- The agarwood qualities of different species under the same inducer as well as different inducers of the same species were be evaluated in this study. The results showed that *A. malaccensis* had better quality than *A. sinensis* and the quality of agarwood induced by FAM71 was better than that of NA8 or FA. The quality of agarwood could be quickly and comprehensively evaluated by gray correlation degree and TOPSIS analysis.



Abbreviations used: GC-MS: gas chromatography mass spectrometry; AMDIS: automatic mass spectral deconvolution and identification system; TOPSIS: technique for order preference by similarity to an ideal solution; AEC: the ethanol-soluble extraction content; OD: overall desirability; FAM71: formic acid combined with Hypocrea jecorina M71; FA: formic acid; *NA8: Nigrospora oryzae A8*; RIs: retention indices; comp.: compound; PCA: principal components analysis; OPLS-DA: orthogonal partial least-squares discrimination analysis; VIP: variable important plot; TCM: traditional Chinese medicine; PECs: 2-(2-phenylethyl) chromones; THPECs: tetrahydro-2-(2-phenylethyl) chromones; EPECs: epoxy-(2-phenylethyl) chromones; DEPECs: diepoxy-(2-phenylethyl) chromones.

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INTRODUCTION

Agarwood, also known as Gaharu in Southeast Asia and Chen Xiang in China, is the dark fragrant resinous heartwood secreted by *Aquilaria* spp. Trees.[1] The chemical composition of agarwood includes sesquiterpenes, chromones, flavonoids, benzophenones, diterpenoids, triterpenoids, and lignans.[2,3] Agarwood has been renowned for its aroma since ancient

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times, mainly due to its sesquiterpenoids.[4] In addition, agarwood has been widely used as an ingredient in traditional herbal medicines for its sedative, carminative, digestive aid, gastropathy, and pain reliever.[5-7] In recent years, growing number of studies have shown that the crude extracts or isolates compounds of agarwood have antiasthma, antioxidant, antimicrobial, antidiabetic, and antiatherosclerosis properties.[8] Furthermore, the agarwood essential oil possesses antiinflammatory and antioxidant properties, as well as having certain effects on the central nervous system.[9-12] The technology of artificial agarwood induction is mainly inspired by the natural agarwood formation process.[13] Compared to natural agarwood, artificial agarwood has a shorter resin production cycle, relatively stable quality, and high yield.[2] In recent years, greater attention has been paid to sustainable planting and management of agarwood to solve the shortage of agarwood in the market, with China and Southeast Asian countries increasing the yield of agarwood through artificial induction.[11,14]

Nowadays, inducers are increasingly being used to produce agarwood in *Aquilaria* trees, which mainly include fungal inoculum and chemical formulations. The effective biological agents that can induce agarwood formation in healthy *Aquilaria* trees are mainly the pure-culture strains of fungi isolated from natural agarwood. Meanwhile, the fungal inoculum is generally considered both safe and eco-friendly.[1] Liu *et al.*[15] analyzed agarwood and fungi from five different parts of the same *Aquilaria* tree, identifying many terpenoids, such as guaiol, agaruspirol, and α-eudesmol using GC-MS. Correlation analysis of the detected compounds with different types of fungi on these five sites indicated that the compounds in agarwood were related to the types of fungi, such as (+)-valencene, which was found to be significantly related to the fungal genus *Thaxteriella*. The chemical inducers generally include phytohormones, salts, minerals, and biological-derived substances.[16,17] A satisfactory yield and good quality can be obtained by applying appropriate inducers with special devices. The chemical composition of agarwood is related to the tree species, inducer, and induction duration. Chen *et al.*[18] investigated the relationship between the expression of chalcone synthase genes and dynamic changes in chromone content in agarwood induced by formic acid stimulation combined with *Fusarium* sp. A2 inoculation. Chromones were not detected until 2 months later, and their content increased with time, peaking at 12 months, which was consistent with the relative gene expression level of *CHS*1 also peaking at 12 months. Sun *et al.*[19] used GC-MS to analyze and identify 232 compounds in agarwood samples from eight different regions across four countries. The sample classification was proven to be regional when combined with factor analysis. Wang *et al.*[20] used GC-MS to examine the chemical constituents of volatile components and ethanol extracts from different organs of *A. sinensis* and agarwood grown in different regions. Sesquiterpenoids, an aromatic species, were discovered as the active ingredients in agarwood from different habitats.

Initially, grading of agarwood quality was mainly based on the characters of color, resin proportion, submerged water or not, as well as smell and shape, all of which were highly subjective. At present, the grade and quality evaluation of agarwood are mainly determined by investigating the content of ethanol-soluble extraction and the color reaction. The Standard Nasional Indonesia of Gaharu (SNI 7631:2011) has five grades based on color, weight, and smell: Double Super, Super A, Super B, Super tanggung (under water), and Super tanggung A (up water).[21] Siti Nazirah Ismail *et al.*[22] used ^{1}H NMR to classify the agarwood from *A. malaccensis*, reporting that agarwood samples with high contents of kusunol, jinkohol. and 10-*Epi*-γ-eudesmol could be reclassified as the "High-Grade" Group, while the "intermediate grade" group was dominated by fatty acids and vanillic acid. The "low-grade" group had higher contents of aquilarone derivatives and phenylethyl chromones.

In previous studies, the majority of agarwood collected for the analysis of agarwood did not indicate the composition of the inducer or the induction mode. The uncertainty of the inducer and induction approaches of artificial agarwood have a distinct impact on the study of the chemical composition of agarwood. As a result, there may be some variation in species identification. In this study, the same inducer (FAM71) was injected into two different tree species (*A. malaccensis* and *A. sinensis*) using be pinhole-infusion technique. In addition, *A. sinensis* trees were stimulated to produce resin by the different inducers (FA, NA8, and FAM71). In order to identify the differential secondary metabolites, agarwood trichloromethane extracts were analyzed using the GC-MS and multivariate statistical analysis method. The gray correlation degree method and TOPSIS were used to synthetically evaluate the quality of agarwood produced by different *Aquilaria* species and inducers. The aim was to provide a basis for the promotion and application of superior quality tree species and efficient agarwood inducers.

MATERIALS AND METHODS

Agarwood materials and reagents

Fifty-eight artificial agarwood samples corresponding to two species were selected and analyzed from a farm in Xinyi, China, as well as two states in Malaysia, Penang, and Kedah. The FAM71 method was used to induce the formation of resinous in 5-year-old matured trees, including the two tree species of *A. malaccensis* and *A. sinensis*, and 50 batches of agarwood samples that were finally collected. In addition, eight batches of agarwood samples from *A. sinensis* were induced by FA or NA8. *H. jecorina* M71 and *N. oryzae* A8 were selected to inoculate the *A. sinensis* trees, while the fungal strains were isolated from *A. sinensis* (Xinyi, China) that was provided by Prof. Zhang (Institute of Microbiology, Guangdong Academy of Sciences) and preserved at the Guangdong Provincial Key Laboratory of Microbiol Culture Collection and Application. The trees were approximately 3–4 m high, more than 10 cm in diameter, and 50–70 cm apart from each other. A drill was used to make a hole that was 0.5 cm in diameter and 4–5 cm deep in the trunks of trees at a height of 1 m. The induced liquid was injected slowly into the xylem of the tree to stimulate resinous secretion.[23] After several months of induction, the trees were harvested and the dark brown resins of artificial agarwood were collected [Figure 1]. The samples were identified as *A. malaccensis* and *A. sinensis* by Prof. Yan (College of Traditional Chinese Medicine, Guangdong Pharmaceutical University, China). The detailed information of the sample is shown in Table 1. Ethanol and trichloromethane (purity >99.0%) were purchased from Guangzhou Chemical Reagent Factory (China). The agarotetrol standard (>98.6% purity) was purchased from the National Institutes for Food and Drug Control, China. The alkane standards (C_{10}–C_{31}) were purchased from AccuStandard Inc. (USA).

Sample preparation

All samples were cut into small pieces and ground into powder using a grinder and then filtered using 50-mesh sieves. The powder samples of agarwood (0.5 g) were extracted with trichloromethane (10 mL, 24 h) at room temperature. The solvent was evaporated in a water bath (65°C) to obtain viscous semisolid masses, which were then dissolved in 2 mL of trichloromethane and stored in a dark, air-tight sealed vial at 4°C.

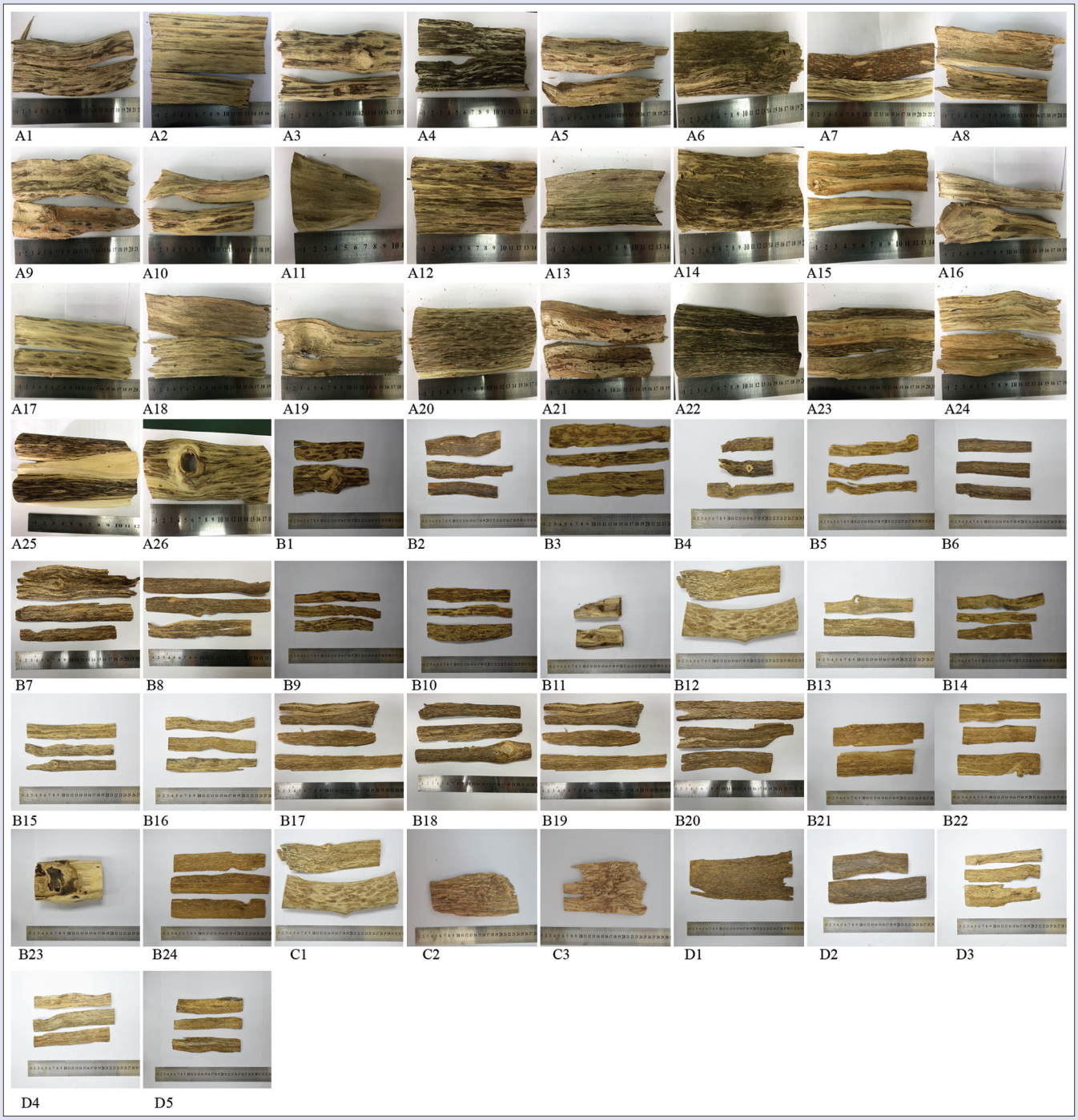

Figure 1: Fifty-eight batches of agarwood samples

Conventional detection

The AEC (%), color reaction, and the content of agarotetrol (%) were tested in accordance with the provisions of *The Pharmacopoeia of People's Republic of China* (2020)[24] [Table 1].

Apparatus and chromatographic conditions

The GC-MS analysis was performed using a GCMS 7890A-5957C (Agilent Technologies) equipped with a capillary fused silica column HP-5MS (30 m × 0.25 mm I.D. × 0.25 μm film thickness, Agilent Technologies). The oven temperature program was initiated at 90°C, held for 4 min before rising at 2.5°C/min to 130°C, maintained for 20 min before rising at 0.5°C/min to 180°C. Following that, the temperature was maintained for 5 min before rising at 2.0°C/min to 200°C. Finally, the temperature increased at 1.0°C/min to 230°C and was maintained for 120 min.

The other operating conditions included the carrier gas, He (99.999%), at a flow rate of 1 mL/min and an injector temperature of 260°C. A solvent delay of 5 min was used, and a 1 μL sample was injected. The samples

Table 1: The detailed information of agarwood samples included in this study and the relevant test results

Index	Source	Inducement method	Brief name	N\E	Collection time	Resin formation time (month)	The content of ethanol-soluble extraction (AEC, %)	The content of agarotetrol (%)	Color-reaction	*R*
A	*A. malaccensis*	Formic acid + *Hypocrea jecorina* M71	A1	5.27°,100.51°	23-07-2016	11	32.5	1.05	Cherry-red	1.43
			A2		24-05-2016	9	31.3	1.31	Cherry-red	3.98
			A3		23-07-2016	11	28.6	0.92	Cherry-red	0.75
			A4		30-03-2016	6	20.5	0.58	Cherry-red	5.83
			A5		25-05-2016	13	20.4	0.42	Cherry-red	1.49
			A6		15-02-2016	8	18.3	0.98	Cherry-red	1.35
			A7		20-03-2016	13	17.1	0.60	Cherry-red	1.27
			A8		24-05-2016	13	16.9	0.51	Cherry-red	3.43
			A9		23-07-2016	11	15.8	0.64	Pale Cherry-red	1.55
			A10		24-05-2016	13	12	0.68	Pale Cherry-red	1.09
			A11		15-02-2016	8	10.9	0.21	Cherry-red	5.51
			A12	5.33°, 100.45°	27-05-2016	9	16.1	0.34	Cherry-red	5.26
			A13		27-05-2016	9	12.5	0.28	Cherry-red	4.07
			A14	5.41°, 100.46°	25-05-2016	9	25	1.00	Cherry-red	3.54
			A15	5.41°, 100.50°	25-05-2016	9	16.3	0.70	Cherry-red	4.79
			A16	5.59°, 100.44°	25-07-2016	11	19	0.45	Pale yellow	2.09
			A17		25-07-2016	11	14.8	0.42	Yellow	3.02
			A18		24-07-2016	11	36.1	2.12	Cherry-red	2.48
			A19		24-07-2016	11	18.6	0.38	Cherry-red	1.12
			A20		27-05-2016	9	13.5	0.37	Pale Brown-yellow	2.19
			A21		24-07-2016	11	11.3	0.39	Pale Cherry-red	2.13
			A22	5.60°,100.41°	24-07-2016	7	19	1.39	Cherry-red	6.82
			A23		30-05-2016	9	10.9	0.53	Cherry-red	8.68
			A24		30-05-2016	9	10.4	0.35	Brown-yellow	6.23
			A25		30-05-2016	9	10.4	1.26	Pale Cherry-red	2.37
			A26	Unknown	27-09-2017	Unknown	14.1	1.50	Cherry-red	2.30
B	*A. sinensis*	Formic acid + *Hypocrea jecorina* M71	B1	22.35°, 110.35°	15-10-2019	12	19.7	1.59	Deep Cherry-red	2.35
			B2		15-07-2013	Unknown	19.4	Undetected	Undetected	3.52
			B3		15-10-2019	12	18	0.50	Deep Cherry-red	3.30
			B4		23-06-2012	12	17.8	Undetected	Cherry-red	2.34
			B5		15-10-2019	12	17.3	0.63	Deep Cherry-red	2.21
			B6		23-06-2012	12	17.3	Undetected	Brown-yellow	1.23
			B7		08-09-2020	24	15.4	1.24	Cherry-red	4.67
			B8		08-09-2020	24	15.3	0.96	Cherry-red	3.73
			B9		15-10-2019	12	15.1	1.81	Pale Cherry-red	2.81
			B10		15-10-2019	12	14.1	1.18	Deep Cherry-red	3.62
			B11		15-07-2013	12	13.9	Undetected	Cherry-red	2.48
			B12		15-07-2013	12	12.7	Undetected	Cherry-red	3.28
			B13		09-01-2010	12	12.7	0.76	Yellow	2.60
			B14		15-10-2019	12	11.9	0.54	Brown	2.23
			B15		15-07-2013	12	10.8	0.71	Cherry-red	4.62
			B16		15-07-2013	12	10.6	0.33	Cherry-red	4.64
			B17		08-09-2020	24	10.6	0.25	Cherry-red	4.42
			B18		08-09-2020	24	10.3	0.65	Cherry-red	4.63
			B19		08-09-2020	24	9.4	0.40	Cherry-red	4.17
			B20		08-09-2020	24	7.7	0.28	Cherry-red	5.15

Contd...

Table 1: Contd...

Index	Source	Inducement method	Brief name	NIE	Collection time	Resin formation time (month)	The content of ethanol-soluble extraction (AEC, %)	The content of agarotetrol (%)	Color-reaction	*R*
			B21		15-07-2013	12	6.0	0.20	Cherry-red	2.91
			B22		15-07-2013	12	6.1	0.16	Cherry-red	7.55
			B23		15-07-2013	12	Undetected	Undetected	Undetected	1.39
			B24		23-06-2012	12	Undetected	Undetected	Undetected	25.07
C		*Nigrospora oryzae* A8	C1		16-12-2011	25	13.9	0.36	Cherry-red	5.38
			C2		16-12-2011	30	12.3	0.92	Cherry-red	3.62
			C3		16-12-2011	30	4.95	0.03	Yellow	1.26
D		Formic acid	D1		24-06-2013	12	36.1	1.94	Cherry-red	2.72
			D2		24-06-2014	12	20.8	2.21	Cherry-red	1.68
			D3		01-07-2011	13	13.7	0.27	Cherry-red	4.46
			D4		24-06-2012	12	11.8	0.57	Cherry-red	0.96
			D5		12-16-2011	12	10.3	2.24	Cherry-red	2.53

were processed using the electron ionization (EI) mode (70 eV). The *m/z* values were recorded in the 50–500 amu range.

The apparent abundance of GC-MS fingerprint (R)

The ratio of sum peak area in the 130–305 min range of individual agarwood to that in the range of 0–130 min was determined as the apparent abundance of GC-MS fingerprints.

Identification of secondary metabolites

The trichloromethane extract of agarwood was analyzed according to the GC-MS conditions, and the GC-MS data were imported into AMDIS software. The components eluting within the total ion chromatogram were extracted using AMDIS, the matrix interference was resolved, and the overlapping components were removed. The mass spectral fragmentation patterns were compared to those stored in the NIST Mass Spectral Library (NIST14), which was built up using pure substances and mass spectra from literature. The RIs of GC on HP-5MS columns were compared to the RI(s) of pure substances in the library. In order to obtain the linear RI values of the volatile compounds, a series of n-alkanes (C_{10}–C_{31}) were run in similar conditions. The chromatographic peaks were confirmed and the chemical components in the chloroform extract of agarwood were identified. A GC-MS and library search could be used to identify the volatile components, while the chromone could be inferred from its fragments and references. The relative percentage content of each component was calculated using the area normalization method. Finally, the retrieval results were summarized and integrated to produce the secondary metabolites identification table.

Multivariate analysis

The relative percentage contents of the 47 retrieved compounds were analyzed by PCA and OPLS-DA using SIMCA-P+ 14 software (Umetrics, Sweden). PCA generated a scores plot that provides a visual determination of similarity among the secondary metabolite profiles. When a new secondary metabolite exhibited unexpected characteristics that differed significantly from the major good secondary metabolite, it was excluded from the model and diagnosed as something different. Therefore, the PCA score plot could distinguish between different categories of samples. After PCA analysis, a more sophisticated OPLS-DA model (the systematic variation of X is divided into two parts: one is linearly related to Y and the other is orthogonal to Y) with specific discriminant information between different groups was obtained. The substitution test was used to verify whether the OPLS-DA model was overfitting, then the V-plot and S-plot were used to analyze significant differences between the agarwood samples from *A. malaccensis* and *A. sinensis*.

Gray relational degree and TOPSIS analysis

The SPSSAU V20.0 online analysis software (https://spssau.com/) was used to analyze TOPSIS and gray correlation degree. The AEC, the content of agarotetrol, R, the relative peak area of 6 groups of common components, and 12 groups of different components were included in the analysis. However, because three compounds were both common components and differential secondary metabolites, so only 18 characters were included in the analysis. Due to the lack of data on the AEC and the content of agarotetrol in 7 out of 58 batches of samples, only 51 batches of samples were available.

The TOPSIS method needed to ensure that the evaluation characters showed all in a positive trend (the greater the value, the better). As agarwood samples with R values of less than or equal to 1 were mostly natural agarwood, the lower the value of R is, the better the quality of agarwood. Therefore, the R fell under the category of low-priority targets. In this study, the counting backward technique was used to

Table 2: Normalized values of 21 characters of 51 batches of agarwood

Index	AEC (%)	The content of agarotetrol (%)	1/R	comp. 6	comp. 11	comp. 12	comp. 16	comp. 20	comp. 23
A1	0.895	0.000	0.019	0.007	0.007	0.012	0.014	0.068	0.012
A2	0.743	0.000	0.006	0.005	0.002	0.001	0.002	0.018	0.214
A3	0.879	0.000	0.041	0.005	0.007	0.018	0.013	0.240	0.025
A4	0.642	0.000	0.005	0.002	0.000	0.000	0.000	0.018	0.101
A5	0.783	0.000	0.026	0.008	0.006	0.016	0.013	0.177	0.084
A6	0.647	0.000	0.026	0.000	0.000	0.000	0.000	0.009	0.461
A7	0.730	0.000	0.034	0.022	0.016	0.019	0.019	0.120	0.055
A8	0.621	0.000	0.011	0.006	0.014	0.017	0.012	0.091	0.094
A9	0.635	0.000	0.026	0.011	0.005	0.009	0.013	0.159	0.052
A10	0.626	0.000	0.048	0.016	0.006	0.008	0.016	0.010	0.027
A11	0.402	0.000	0.007	0.010	0.001	0.001	0.003	0.055	0.165
A12	0.584	0.000	0.007	0.005	0.000	0.000	0.000	0.032	0.064
A13	0.476	0.000	0.009	0.006	0.000	0.000	0.000	0.046	0.092
A14	0.730	0.000	0.008	0.007	0.003	0.001	0.002	0.049	0.072
A15	0.552	0.000	0.007	0.001	0.001	0.001	0.001	0.026	0.360
A16	0.707	0.000	0.018	0.001	0.005	0.005	0.007	0.106	0.039
A17	0.601	0.000	0.013	0.007	0.019	0.003	0.006	0.073	0.043
A18	0.818	0.000	0.009	0.008	0.005	0.011	0.009	0.104	0.052
A19	0.830	0.000	0.040	0.012	0.024	0.048	0.037	0.424	0.082
A20	0.604	0.000	0.020	0.003	0.003	0.005	0.005	0.125	0.038
A21	0.583	0.000	0.024	0.005	0.009	0.006	0.012	0.123	0.067
A22	0.506	0.000	0.004	0.001	0.000	0.000	0.001	0.019	0.294
A23	0.343	0.000	0.004	0.005	0.000	0.000	0.000	0.019	0.116
A24	0.310	0.000	0.005	0.001	0.000	0.000	0.000	0.011	0.673
A25	0.455	0.001	0.019	0.009	0.000	0.002	0.002	0.074	0.048
A26	0.572	0.001	0.018	0.004	0.003	0.000	0.005	0.088	0.087
B1	0.653	0.001	0.014	0.003	0.011	0.005	0.006	0.056	0.159
B3	0.546	0.000	0.009	0.000	0.003	0.000	0.000	0.025	0.238
B5	0.592	0.000	0.015	0.000	0.009	0.000	0.000	0.037	0.142
B7	0.533	0.000	0.007	0.000	0.000	0.000	0.000	0.022	0.124
B8	0.503	0.000	0.009	0.000	0.000	0.000	0.000	0.021	0.100
B9	0.512	0.001	0.012	0.006	0.003	0.000	0.004	0.042	0.102
B10	0.518	0.000	0.010	0.002	0.005	0.005	0.008	0.103	0.115
B13	0.437	0.000	0.013	0.000	0.000	0.000	0.000	0.019	0.048
B14	0.412	0.000	0.016	0.003	0.000	0.000	0.000	0.064	0.048
B15	0.385	0.000	0.008	0.000	0.000	0.000	0.000	0.010	0.699
B16	0.356	0.000	0.007	0.000	0.000	0.000	0.000	0.023	0.586
B17	0.373	0.000	0.008	0.000	0.000	0.000	0.000	0.013	0.142
B18	0.363	0.000	0.008	0.000	0.000	0.000	0.000	0.023	0.107
B19	0.540	0.000	0.014	0.000	0.000	0.000	0.000	0.018	0.151
B20	0.309	0.000	0.008	0.000	0.000	0.000	0.000	0.009	0.123
B21	0.188	0.000	0.011	0.000	0.000	0.000	0.000	0.044	0.075
B22	0.162	0.000	0.004	0.000	0.000	0.000	0.000	0.006	0.729
C1	0.499	0.000	0.007	0.001	0.001	0.008	0.008	0.099	0.066
C2	0.601	0.000	0.013	0.003	0.000	0.004	0.005	0.056	0.010
C3	0.334	0.000	0.054	0.004	0.001	0.002	0.009	0.342	0.016
D1	0.790	0.000	0.008	0.000	0.000	0.002	0.003	0.084	0.131
D2	0.661	0.001	0.019	0.001	0.000	0.011	0.009	0.137	0.194
D3	0.700	0.000	0.011	0.000	0.000	0.007	0.012	0.499	0.049
D4	0.381	0.000	0.034	0.001	0.000	0.009	0.010	0.089	0.090
D5	0.264	0.001	0.011	0.001	0.000	0.009	0.014	0.216	0.049

Index	comp. 27	comp. 28	comp. 30	comp. 31	comp. 33	comp. 34	comp. 38	comp. 43	comp. 47
A1	0.017	0.025	0.184	0.285	0.236	0.014	0.134	0.000	0.051
A2	0.018	0.035	0.320	0.207	0.434	0.025	0.256	0.010	0.021
A3	0.015	0.028	0.145	0.283	0.150	0.000	0.163	0.000	0.038
A4	0.027	0.069	0.367	0.300	0.410	0.049	0.418	0.000	0.047
A5	0.033	0.012	0.208	0.468	0.244	0.039	0.117	0.045	0.043
A6	0.008	0.008	0.225	0.155	0.471	0.015	0.265	0.024	0.006
A7	0.025	0.049	0.266	0.311	0.442	0.079	0.262	0.000	0.050
A8	0.042	0.047	0.342	0.387	0.480	0.089	0.278	0.077	0.053
A9	0.032	0.063	0.240	0.518	0.332	0.058	0.347	0.000	0.048
A10	0.049	0.000	0.253	0.609	0.304	0.096	0.250	0.000	0.042
A11	0.046	0.083	0.344	0.134	0.763	0.000	0.265	0.000	0.100
A12	0.026	0.074	0.222	0.360	0.497	0.095	0.457	0.000	0.055

Contd...

Table 2: Contd...

Index	comp. 27	comp. 28	comp. 30	comp. 31	comp. 33	comp. 34	comp. 38	comp. 43	comp. 47
A13	0.027	0.060	0.459	0.366	0.510	0.057	0.372	0.000	0.093
A14	0.037	0.079	0.271	0.369	0.445	0.000	0.205	0.000	0.005
A15	0.024	0.054	0.214	0.257	0.628	0.116	0.203	0.000	0.018
A16	0.026	0.054	0.388	0.279	0.338	0.067	0.350	0.000	0.114
A17	0.043	0.063	0.410	0.412	0.422	0.062	0.306	0.040	0.096
A18	0.027	0.011	0.329	0.305	0.303	0.014	0.145	0.022	0.023
A19	0.015	0.000	0.212	0.130	0.223	0.028	0.051	0.000	0.043
A20	0.026	0.044	0.358	0.313	0.369	0.039	0.451	0.000	0.148
A21	0.057	0.030	0.281	0.547	0.285	0.125	0.372	0.000	0.105
A22	0.022	0.062	0.479	0.273	0.545	0.037	0.224	0.000	0.015
A23	0.029	0.063	0.481	0.347	0.535	0.019	0.471	0.000	0.058
A24	0.018	0.036	0.188	0.158	0.574	0.038	0.239	0.000	0.028
A25	0.031	0.062	0.311	0.476	0.422	0.052	0.514	0.000	0.104
A26	0.026	0.045	0.498	0.446	0.383	0.063	0.219	0.000	0.054
B1	0.025	0.051	0.193	0.425	0.329	0.000	0.447	0.057	0.056
B3	0.000	0.029	0.506	0.223	0.302	0.000	0.489	0.000	0.043
B5	0.010	0.036	0.207	0.350	0.245	0.012	0.621	0.061	0.091
B7	0.026	0.000	0.229	0.427	0.352	0.025	0.577	0.037	0.039
B8	0.012	0.049	0.174	0.385	0.289	0.036	0.670	0.041	0.130
B9	0.014	0.054	0.178	0.508	0.344	0.000	0.552	0.070	0.070
B10	0.008	0.045	0.364	0.348	0.424	0.041	0.503	0.045	0.120
B13	0.000	0.004	0.061	0.450	0.204	0.033	0.693	0.171	0.050
B14	0.000	0.029	0.210	0.345	0.242	0.024	0.762	0.055	0.104
B15	0.011	0.033	0.013	0.210	0.121	0.021	0.542	0.037	0.084
B16	0.007	0.032	0.389	0.163	0.450	0.023	0.366	0.025	0.115
B17	0.053	0.000	0.195	0.273	0.410	0.061	0.722	0.046	0.146
B18	0.013	0.053	0.363	0.300	0.296	0.039	0.719	0.043	0.140
B19	0.021	0.084	0.286	0.467	0.513	0.065	0.106	0.087	0.277
B20	0.000	0.000	0.205	0.246	0.375	0.066	0.776	0.037	0.197
B21	0.000	0.000	0.406	0.205	0.154	0.052	0.838	0.000	0.147
B22	0.000	0.025	0.344	0.058	0.438	0.030	0.353	0.000	0.059
C1	0.000	0.000	0.144	0.228	0.353	0.077	0.723	0.017	0.023
C2	0.000	0.003	0.110	0.192	0.353	0.000	0.535	0.000	0.397
C3	0.000	0.000	0.024	0.179	0.359	0.008	0.433	0.018	0.467
D1	0.000	0.000	0.177	0.387	0.268	0.008	0.303	0.018	0.071
D2	0.000	0.049	0.037	0.189	0.648	0.029	0.148	0.135	0.027
D3	0.000	0.022	0.022	0.263	0.269	0.047	0.303	0.021	0.087
D4	0.000	0.068	0.104	0.409	0.331	0.000	0.708	0.000	0.062
D5	0.000	0.000	0.181	0.253	0.000	0.000	1.000	0.000	0.757

transform low-priority targets into high-priority targets. The formula was used to carry on normalization processing of the data, to obtain the normalization matrix value.[25] In formula 1, "*n*" was the number of indices, "Xij" represented the value of the *i*th sample on the *j*th character and "aij" represented the normalized value of the *i*th sample on the *j*th evaluation character. The matrix was then imported into the SPSSAU software for TOPSIS analysis [Table 2]. The positive and negative ideal solution distances, D^+ and D^-, determined, followed by the relative proximity *c*i, which represented the degree to which the evaluation object is close to the optimal scheme.

$$a_{ij} = \frac{X}{\sqrt{\sum_{i=1}^{n} X_{ij}^2}} \quad \text{(Formula 1)}$$

However, according to TOPSIS, *c*i could only reflect the internal relative closeness of each evaluation object, and it was necessary to analyze the gray correlation degree. The gray correlation degree was mainly through selecting the best quality of each character from 51 batches of agarwood to serve as the ideal sample. Based on the ideal sample position, the correlation coefficient between each character of the sample and the ideal sample was calculated. The data were analyzed using the SPSSAU software to obtain *r*i values. The geometric mean of the *c*i and *r*i values was calculated to obtain the OD using formula 2. In formula 2, the "X" represented the character included in the investigation and "*k*" represented the number of characters.

$$OD = (X_1 + X_2 + ... + X_k) / k \quad \text{(Formula 2)}$$

RESULTS

Analysis of secondary metabolites

The GC-MS original data were converted into corresponding format files and imported into the AMDIS software to retrieve each peak. The components that eluted in the total ion chromatogram were extracted in AMDIS. The secondary metabolites were identified by comparing their mass spectra and retention index with those of commercial standards. Finally, the results were sorted into a total secondary metabolite retrieval table (see supplementary materials for details). A total of 47 secondary metabolites were retrieved, including 17 sesquiterpenes, 24 2-(2-phenylethyl) chromone compounds, 4 aromatic acids, and 2 fatty acid compounds. There were 6 common peaks in 58 batches of samples [Table 3], with 12 common peaks detected in 26 batches of *A. malaccensis* and 6 common peaks detected in 32 batches of *A. sinensis*. The similarity evaluation system for the chromatographic fingerprint of TCM (2004 A) was used to overlap the GC-MS chromatogram [Figure 2a and 2b].

Under the same FAM71 inducer, the total contents of aromatic compounds and sesquiterpenes in *A. malaccensis* were higher than in *A. sinensis*, but the total content of 2-(2-phenylethyl) chromone compounds

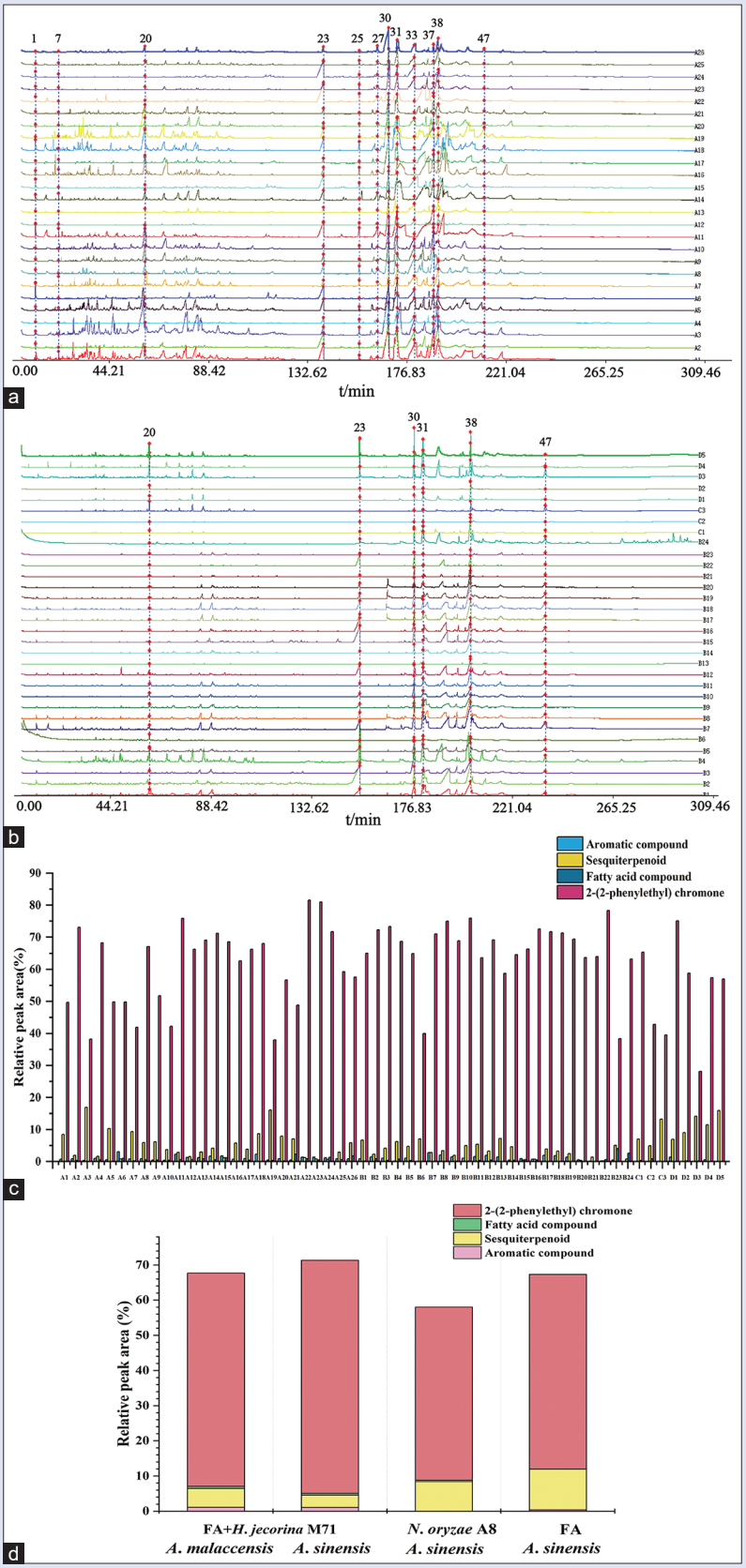

Figure 2: Overlapped GC-MS chromato gram for 26 batches of agarwood in *A. malaccensis* (A1-A26) (a), 32 batches of agarwood in *A. sinensis* (B1–D5) (b), Box plot of 58 (c), and the Percentiles (d) of the relative content of four different types of compounds in 58 samples

were lower than in *A. sinensis* [Figure 2c and d]. The relative content of sesquiterpenoids in *A. malaccensis* was 53% higher than in *A. sinensis*. The top seven relative contents of sesquiterpenoids were all *A. malaccensis*, with sample A3 ranking highest and having the lowest R-value (R = 0.75). The total relative content of 2-(2-phenylethyl) chromone compounds in *A. sinensis* was 10% compared to *A. malaccensis*, and the R-value of the B24 sample of *A. sinensis* was the highest (R = 25.07).

In the same species of *A. sinensis*, the total relative content of sesquiterpenoids in agarwood induced by FA was the highest, followed by the NA8 and the lowest was FAM71 [Figure 2c and d]. The number of sesquiterpenoids in *A. sinensis* induced by FA was 37% higher than in agarwood induced by NA8 and was three times that of agarwood induced by FAM71. The total content of chromone compounds in *A. sinensis* induced by FAM71 was 35% higher than in NA8 induced agarwood and 20% higher than in FA induced agarwood.

Multivariate statistical analysis of differential secondary metabolites

The PCA score plot reveals several trends [Figure 3a]. According to the *X*-axis, *A. malaccensis* and *A. sinensis* can be separated even with the same FAM71 inducement method. There was a tendency to separate the samples of NA8 inoculation (C samples), FA stimulation (D samples), and FAM71 (B samples). In addition, the 26 batches of *A. malaccensis* agarwood samples were also found to be divided into two groups by the *Y*-axis. In addition to A20 (resin formation time of 9 months) and A26 (resin formation time is unknown), the resin formation time of *A. malaccensis* in the negative half of the *Y*-axis was more than or equal to 11 months. In the positive half of the *Y*-axis, the resin formation time of *A. malaccensis* was less than 11 months.

The OPLS-DA model was established to further understand the difference in agarwood between *A. malaccensis* and *A. sinensis* [Figure 3b]. The 58

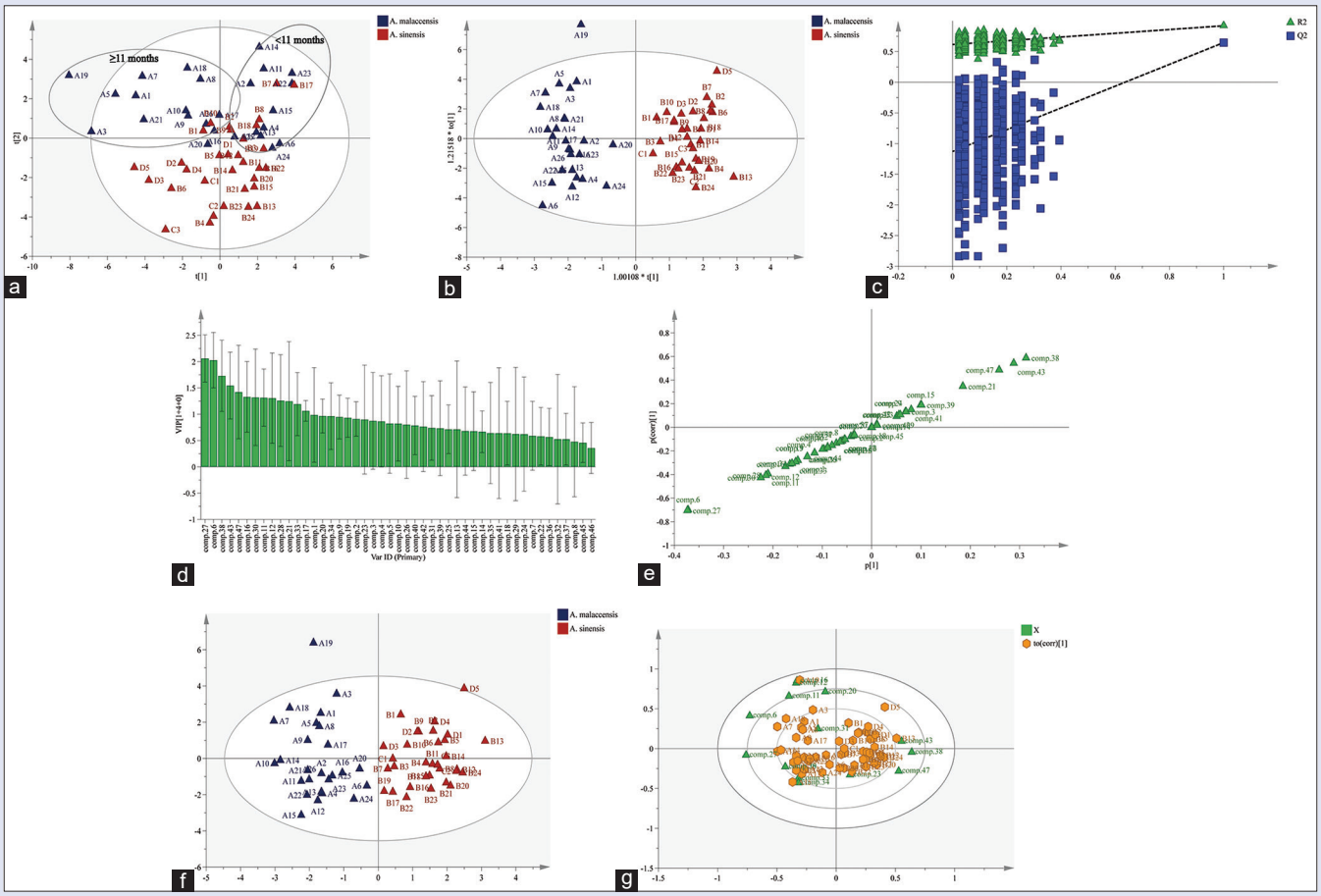

Figure 3: PCA score plots of 47 secondary metabolites (a), OPLS-DA results (b), Permutation test (c), Variable Important Plot (VIP > 1.00) (d), S-plot from OPLS-DA (e), OPLS-DA scores plots (f), and Biplot (g) of 15 secondary metabolites representing 58 batches of samples

Table 3: Identification of common components of fingerprints from 58 batches of agarwood samples

Comp.	t/min[a]	Chemical name[b]	Formula	CAS#	RI[c]	Literature
20	62.530	Baimuxinal	$C_{15}H_{24}O_2$	86408-21-1	1820.0	[13]
23	144.414	2-(2-phenylethyl) chromone	$C_{17}H_{14}O_2$	61828-53-3	2297.0	[18]
30	170.476	6-methoxy-2-(2-phenylethyl) chromone	$C_{18}H_{16}O_3$	\	2580.3	[13]
31	173.334	5,8-dihydroxy-2-[2-(4-methoxyphenyl) ethyl] chromone	$C_{18}H_{16}O_5$	128922-70-3	2981.3	[13]
38	192.817	6-methoxy-2-[2-(3-methoxyphenyl) ethyl] chromone	$C_{19}H_{18}O_4$	\	2890.8	[18]
47	223.747	6,7-dimethoxy-2-[2-(4-methoxyphenyl) ethyl] chromone	$C_{20}H_{20}O_5$	117596-92-6	3459.0	[26]

"\" not determined. [a]Average retention times (min) for main identified components. [b]Identification was made according to comparison of resolved mass spectra with those of standards in MS Library Database. [c]Kovats retention indices in HP-5MS column in reference to normal alkanes

agarwood samples were divided into two groups based on *A. malaccensis* and *A. sinensis*, with these two groups clustering well and separating significantly. There were three mode parameters for the OPLS-DA model: R^2X (cum) and R^2Y (cum) represented the explanatory ability of the OPLS-DA model's principal component to variables in the direction of *X*-axis or *Y*-axis, respectively, while Q^2 (cum) represented the predictive ability of the OPLS-DA model for grouping. The closer the three values were to one, the stronger the explanatory and prediction of the model. In this model, two principal significant components described 38.4% of the variation in X (R^2X = 0.384), 92.2% of the variation in Y (R^2Y = 0.922), and predicted 64.7% (Q^2(cum) = 0.647) according to cross-validation. Therefore, this revealed that the grouping model was capable of interpretation and prediction, and the clustering results were reliable. The permutation test was used for internal validation of the model to prevent overfitting of the model [Figure 3c]. Generally, the Q^2 and R^2 values of the model must to be validated. When the intercept of the result of the permutation test on the *y axis* did not exceed 0.05 ($Q^2 < 0.05$), the model could be considered as not overfitted. After 800 permutation tests, the intercept values of R^2 and Q^2 were 0.614 and -1.13, respectively, all the R^2 and Q^2 values on the left were lower than the rightmost value, and the intercept of the regression curve of Q^2 was less than 0.00. These indicated that the established OPLS-DA model established was not overfitted and had good predictive ability.

The variable importance in the projection method was used to determine the significant differential secondary metabolites that were differentially produced between the agarwood from *A. malaccensis* and *A. sinensis*. A VIP was used to select the significant secondary metabolites that were differentially produced between *A. malaccensis* and *A. sinensis* [Figure 3d]. A VIP value greater than 1.00 was used as the screening index and 13 differential components were obtained. Moreover, in order to observe the contribution rate of variables in the model to grouping, an S-plot of the relative peak area of all secondary metabolites was generated using the OPLS-DA model [Figure 3e]. The ordinate *P* (corr) in the S-plot represented the correlation coefficient of each component. The further the component was from the origin, the greater its contribution to grouping. Among them, compounds 27 [*P* (corr) = -0.705], 6 [*P* (corr) = -0.701], 30 [*P* (corr) = -0.424], 43 [*P* (corr) = 0.542], and 38 [*P* (corr) = 0.590] contributed to more than 58 batches of samples. Combined with VIP and S-plot, 12 secondary metabolites representing the differences between the samples of *A. malaccensis* and *A. sinensis* were screened.

However, 3 of the 12 secondary metabolites screened [Table 4] were also presented in the six common components, resulting in 15 secondary metabolites that represented 58 batches of samples and were then verified using OPLS-DA again [Figure 3f]. The parameters of the OPLS-DA model established in this study were R^2X (cum) = 0.653, R^2Y (cum) = 0.833, and Q^2(cum) = 0.708, indicating that the grouping model had strong interpretation and prediction ability, and the clustering results were reliable. Following that, the Biplot [Figure 3g] was generated using the OPLS-DA model and the 58 sample batches were grouped into two groups based on different tree species. The distribution positions of different components in the Biplot revealed their corresponding contribution rates to the grouping of the two groups of samples. Compounds 6, 11, 12, 16, 20, 27, 28, 30, 31, 33, and 34 were grouped with samples from *A. malaccensis*, which contributed the most to the grouping. In addition, compounds 23, 38, 43, and 47 were clustered together with *A. sinensis* and contributed the most to the grouping.

Analysis of differential secondary metabolites

In order to prove the differences between *A. malaccensis* and *A. sinensis*, as well as to compare the secondary metabolites in agarwood induced by FAM71, NA8, and FA, 12 differential secondary metabolites were divided into two parts: nonchromone compounds and the 2-(2-phenylethyl) chromone [Figure 4].

The relative peak area of sesquiterpenes in *A. malaccensis* was higher than *A. sinensis* using the same FAM71 inducer [Figure 4a]. The contents of α-cedrene epoxide and agarospirol in *A. malaccensis* were eight times that of *A. sinensis*, whereas the contents of 10-*Epi*-γ-eudesmol and (-)-aristolene in *A. malaccensis* were two and four times those of in *A. sinensis*, respectively. Compared to the three different inducement methods in *A. sinensis*, the relative peak areas of agarospirol and (-)-aristolene were the highest in *A. sinensis* induced by FA, which was twice that of NA8 inoculation, and three times that of FAM71. The relative peak areas of α-Cedrene epoxide and 10-*Epi*-γ-Eudesmol in agarwood induced by NA8 and FAM71 were 10 and 40 times than the FA method, respectively.

There were 8 chromone compounds among the 12 different components, with 6-methoxy-2-(2-phenylethyl) chromone (comp. 30), 7-hydroxy-2-(2-phenylethyl) chromone (comp. 33), and 6-methoxy-2-[2-(3-methoxyphenyl) ethyl] chromone (comp. 38) accounting for a large proportion in all samples [Figure 4b]. Using the same FAM71 inducer, the relative contents of compound 30 and 33 in *A. malaccensis* were 41% and 26% higher than those in *A. sinensis*, respectively. However, the relative content of compound 38 in *A. malaccensis* was 49% lower than in *A. sinensis*. The relative percentages of the first five differential secondary metabolites were all higher than those of *A. sinensis*, while the last three differential secondary metabolites were lower. A comparison of different inducers in *A. sinensis* revealed that the

Table 4: Identification of differential secondary metabolites from 58 batches of agarwood samples

Comp.	t/min[a]	Chemical name[b]	Formula	CAS#	RI[c]	Literature
6	22.279	α-Cedrene epoxide	$C_{15}H_{24}O$	29597-36-2	1569.0	[27]
11	33.342	10-*Epi*-γ-Eudesmol	$C_{15}H_{26}O$	15051-81-7	1619.0	[22]
12	35.223	Agarospirol	$C_{15}H_{26}O$	1460-73-7	1646.0	[18]
16	36.180	(-)-Aristolene	$C_{15}H_{24}$	6831-16-9	1403.0	[27]
27	164.070	2-[2-hydroxy-2-(4-hydroxyphenyl) ethyl] chromone	$C_{17}H_{14}O_4$	\	\	[27]
28	164.639	6-hydroxy-2-(2-phenylethyl) chromone	$C_{17}H_{14}O_3$	84294-90-6	2729.3	[13]
30	170.476	6-methoxy-2-(2-phenylethyl) chromone	$C_{18}H_{16}O_3$	\	2580.3	[13]
33	182.214	7-hydroxy-2-(2-phenylethyl) chromone	$C_{17}H_{14}O_3$	\	\	[27]
34	186.165	6-methoxy-2-[2-(4-hydroxyphenyl) ethyl] chromone	$C_{18}H_{16}O_4$	\	\	[27]
38	192.817	6-methoxy-2-[2-(3-methoxyphenyl) ethyl] chromone	$C_{19}H_{18}O_4$	\	2890.8	[18]
43	198.999	5-hydroxy-6-methoxy-2-(2-phenylethyl) chromone	$C_{18}H_{16}O_4$	\	2779.1	[18]
47	223.747	6,7-dimethoxy-2-[2-(4-methoxyphenyl) ethyl] chromone	$C_{20}H_{20}O_5$	117596-92-6	3459.0	[26]

"\"not determined. [a]Average retention times (min) for main identified components. [b]Identification was made according to comparison of resolved mass spectra with those of standards in MS Library Database. [c]Kovats retention indices in HP-5MS column in reference to normal alkanes

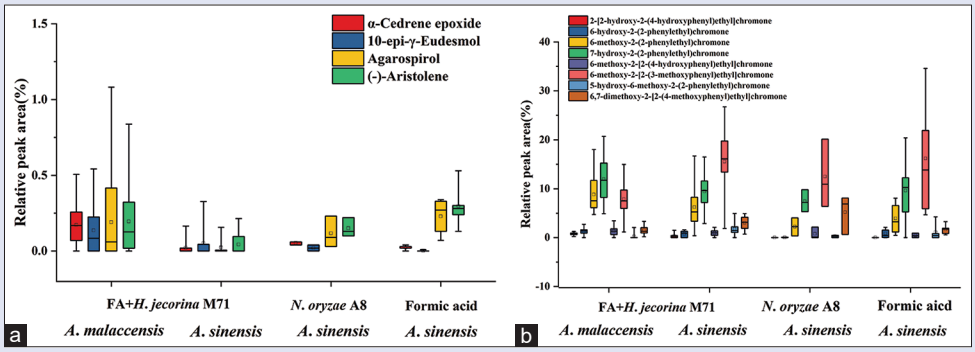

Figure 4: Box-plot of four nonchromone compounds in different components (a). Box-plot of eight chromone compounds in different components (b)

relative percentage of compound 30 in the FAM71-induced agarwood was three times that of the agarwood induced by NA8 and 1.6 times that of the FA-induced agarwood. The relative percentages of compounds 33 and 38 in the FA-induced agarwood were 29% higher than those in the NA8-induced agarwood and similar to those in the FAM71-induced agarwood.

Analysis of TOPSIS and gray correlation degree

According to the TOPSIS analysis, the top five samples with the highest *ci* values were all FAM71-induced agarwood samples, with the first four samples all from *A. malaccensis* [Table 5]. The *ci* values of the three batches of samples induced by NA8 ranked third, sixth, and seventh from the bottom. The FA-induced agarwood sample D5 ranked last out of the 51 batches of samples. The larger the *ci* value was, the closer it was to the ideal sample. Under the same inducer FAM71 conditions, the agarwood samples from *A. malaccensis* were closer to the ideal sample than *A. sinensis*. For *A. sinensis*, the agarwood induced by FAM71 was closer to the ideal samples than those induced by NA8 or FA.

The correlation coefficient reflects the degree of coincidence between each character and the ideal value. The average of each character is taken as the correlation degree (r_i). The larger the correlation coefficient, the closer it is to the ideal value. In the gray correlation degree ranking, all 26 batches of samples induced by FAM71 from *A. malaccensis* ranked in the top 26, with A10 ranking first. All 17 batches of *A. sinensis* induced by FAM71 ranked between 27 and 43. The remaining samples were all in the bottom eight, including three batches of NA8-induced samples and five batches of FA-induced samples with D3 ranking last. Using the same FAM71 inducer, the close degree of each character to the optimal character of *A. malaccensis* was higher than that *A. sinensis*, indicating that *A. malaccensis* had better quality than *A. sinensis*. For *A. sinensis*, agarwood induced by FAM71 had better quality than that induced by NA8 or FA.

As the values of *ri* and *ci* differed in order, the OD value was used to represent the overall desirability of *ri* and *ci*. The top 33 agarwood samples based on OD value were all induced by FAM71, with the top 23 agarwood samples all from *A. malaccensis*, whereas the FA-induced sample D5 had the lowest OD value. Overall, *A. malaccensis* had better quality than *A. sinensis* and the quality of agarwood induced by FAM71 was better than that of NA8 or FA.

DISCUSSION

At present, various inducers are used in the production of agarwood.[1] It is known that *Trichoderma* is an effective fungus that can produce agarwood.[28] In addition, when compared to chemical method or fungi inoculation method alone, the quality of agarwood obtained by the combination of chemical reagent stimulation combined with fungal inoculation was closer to that of natural agarwood.[13] Therefore, the FAM71 inducer was used to induce the agarwood in this study. The same compounds, such as baimuxinal and 2-(2-phenylethyl) chromone, were detected in different tree species.

Previous studies on agarwood identification mainly focused on the accumulation of certain sesquiterpenes and PECs in high-quality agarwood.[29] Sesquiterpenes, including benzylacetone, agarospirol, hinesol, (-)-aristolene, guaiol, baimuxinal, and others, cannot be detected in healthy *Aquilaria* trees, but only in the early stage of cell death in callus.[28,30] Sesquiterpenes are one of the main pharmacological active components of agarwood, with central inhibitory, sedative, hypnotic, and stomach-strengthening effects.[31-33] There were four sesquiterpenes among the 12 different components, with Agarospirol being the main component of high-grade agarwood that is internationally recognized. Agarospirol and hinesol are mutually opposite isomers that have antigastric ulcer properties and can improve cerebral blood circulation.[34] In addition, some studies have shown that the higher the content of 10-*Epi*-γ-eudesmol, the higher the quality grade of agarwood.[22] The special aroma of agarwood mainly depends on sesquiterpenoids, but PECs with high boiling points also enhance the stability of the aroma. Furthermore, PECs degrade into aromatic compounds when agarwood is heated; therefore, chromone compounds contribute significantly to the aroma and its duration when heated. The chromone compounds in agarwood are grouped into four types according to their backbone structures: THPECs, EPECs, DEPECs, and PECs.[2] The GC-MS used in this study was only capable of detecting PECs. A comparison by Yang *et al.*[35] on the quality of agarwood produced from China and Southeast Asian countries revealed that the AEC and total chromone in *A. sinensis* were both higher than the five agarwood production areas in Southeast Asian countries, including *A. crassna* and *A. khasiana*. Yan *et al.*[29] analyzed agarwood obtained through four different induction methods and discovered that the content of sesquiterpene was the highest in the agarwood obtained by wounding using an axe. Furthermore, the relative content of PECs in the agarwood obtained by both the fungus induction and chemical methods exceeded 60%. At present, the "Qi Nan" agarwood is considered to be high-quality natural agarwood in the agarwood industry, with the sum of the relative contents of 2-(2-phenylethyl) chromone and 2-[2-(4-methoxybenzyl) ethyl] chromone of 51.57–84.71%.[36] In this study, the relative contents of PECs in agarwood induced by FAM71 were all greater than 60% (60.6% for *A. malaccensis* and 66.3% for *A. sinensis*). The relative contents of PECs in agarwood induced by NA8 and FA were 49.2 and 55.3%, respectively, which were lower than those obtained using the comprehensive method of FAM71.

Ethanol can extract a large number of chromone and sesquiterpene compounds from agarwood. According to the local standard of Hainan

Table 5: TOPSIS evaluated the calculation results (*ci*), correlation coefficient results (*ri*), OD results, and the ranking of the respective result values

Item	Index	TOPSIS evaluation and calculation results				Correlation coefficient results							
		D^+	D^-	c_i	Rank (*ci*)	AEC	Agarotetrol content (%)	1/R	comp. 6	comp. 11	comp. 12	comp. 17	comp. 20
1	A1	1.508	1.609	0.516	11	0.449	0.965	0.991	0.975	0.975	0.981	0.984	0.949
2	A2	1.459	1.472	0.502	15	0.571	0.682	0.687	0.686	0.683	0.683	0.684	0.696
3	A3	1.430	1.582	0.525	9	0.750	0.562	0.579	0.564	0.565	0.569	0.567	0.679
4	A4	1.610	1.396	0.464	31	0.984	0.550	0.552	0.551	0.550	0.550	0.550	0.557
5	A5	1.140	1.651	0.592	1	0.944	0.538	0.546	0.541	0.540	0.543	0.542	0.596
6	A6	1.595	1.458	0.478	25	0.847	0.527	0.535	0.527	0.527	0.527	0.527	0.530
7	A7	1.503	1.470	0.494	17	0.789	0.517	0.525	0.522	0.521	0.521	0.521	0.548
8	A8	1.193	1.643	0.579	2	0.761	0.506	0.509	0.508	0.510	0.511	0.510	0.532
9	A9	1.557	1.406	0.474	27	0.716	0.497	0.503	0.499	0.498	0.499	0.500	0.538
10	A10	1.641	1.434	0.466	28	0.720	0.930	0.962	0.941	0.934	0.935	0.941	0.937
11	A11	1.744	1.448	0.454	34	0.766	0.897	0.903	0.906	0.898	0.899	0.900	0.947
12	A12	1.642	1.359	0.453	35	0.680	0.867	0.873	0.871	0.867	0.867	0.867	0.894
13	A13	1.588	1.473	0.481	24	0.775	0.839	0.845	0.843	0.839	0.839	0.839	0.873
14	A14	1.775	1.414	0.443	41	0.575	0.812	0.819	0.818	0.814	0.813	0.814	0.857
15	A15	1.645	1.437	0.466	29	0.735	0.787	0.792	0.787	0.788	0.788	0.788	0.806
16	A16	1.543	1.498	0.493	18	0.701	0.764	0.775	0.764	0.766	0.766	0.768	0.835
17	A17	1.359	1.553	0.533	7	0.817	0.741	0.748	0.745	0.751	0.743	0.744	0.782
18	A18	1.288	1.646	0.561	3	0.496	0.721	0.729	0.728	0.725	0.731	0.728	0.828
19	A19	1.412	1.651	0.539	4	0.773	0.701	0.718	0.706	0.711	0.722	0.717	0.948
20	A20	1.565	1.418	0.475	26	0.984	0.664	0.672	0.665	0.665	0.666	0.666	0.716
21	A21	1.510	1.441	0.488	20	0.907	0.648	0.655	0.649	0.650	0.649	0.651	0.689
22	A22	1.653	1.423	0.462	32	0.867	0.632	0.634	0.632	0.631	0.631	0.632	0.643
23	A23	1.704	1.390	0.449	39	0.836	0.616	0.618	0.619	0.616	0.616	0.616	0.626
24	A24	1.694	1.449	0.461	33	0.797	0.602	0.604	0.602	0.602	0.602	0.602	0.607
25	A25	1.663	1.364	0.451	37	0.773	0.588	0.594	0.591	0.588	0.589	0.589	0.612
26	A26	1.498	1.548	0.508	12	0.841	0.575	0.580	0.576	0.576	0.575	0.576	0.604
27	B1	1.305	1.510	0.537	5	0.780	0.487	0.491	0.488	0.490	0.489	0.489	0.503
28	B3	1.646	1.348	0.450	38	0.550	0.397	0.398	0.397	0.397	0.397	0.397	0.402
29	B5	1.488	1.382	0.481	23	0.531	0.390	0.393	0.390	0.392	0.390	0.390	0.397
30	B7	1.445	1.360	0.485	22	0.500	0.385	0.386	0.385	0.385	0.385	0.385	0.388
31	B8	1.620	1.291	0.444	40	0.490	0.379	0.380	0.379	0.379	0.379	0.379	0.383
32	B9	1.451	1.473	0.504	13	0.479	0.373	0.375	0.374	0.374	0.373	0.374	0.380
33	B10	1.428	1.431	0.500	16	0.649	0.478	0.481	0.479	0.479	0.479	0.480	0.504
34	B13	1.575	1.499	0.488	21	0.612	0.469	0.473	0.469	0.469	0.469	0.469	0.474
35	B14	1.637	1.282	0.439	42	0.587	0.461	0.465	0.462	0.461	0.461	0.461	0.477
36	B15	1.682	1.383	0.451	36	0.560	0.453	0.454	0.453	0.453	0.453	0.453	0.455
37	B16	1.515	1.537	0.503	14	0.545	0.445	0.446	0.445	0.445	0.445	0.445	0.450
38	B17	1.642	1.275	0.437	43	0.534	0.437	0.439	0.437	0.437	0.437	0.437	0.440
39	B18	1.655	1.275	0.435	44	0.520	0.430	0.431	0.430	0.430	0.430	0.430	0.435
40	B19	1.507	1.457	0.492	19	0.500	0.423	0.424	0.423	0.423	0.423	0.423	0.425
41	B20	1.780	1.208	0.404	48	0.475	0.416	0.417	0.416	0.416	0.416	0.416	0.417
42	B21	1.978	1.173	0.372	50	0.453	0.409	0.411	0.409	0.409	0.409	0.409	0.419
43	B22	1.762	1.535	0.466	30	0.446	0.403	0.404	0.403	0.403	0.403	0.403	0.404
44	C1	1.612	1.134	0.413	45	0.460	0.368	0.369	0.368	0.368	0.369	0.369	0.383
45	C2	1.819	1.202	0.398	49	0.439	0.363	0.364	0.363	0.363	0.363	0.363	0.369
46	C3	1.782	1.241	0.411	46	0.385	0.358	0.362	0.358	0.358	0.358	0.358	0.385
47	D1	1.376	1.546	0.529	8	0.703	0.353	0.355	0.353	0.353	0.353	0.353	0.372
48	D2	1.381	1.593	0.536	6	0.485	0.348	0.351	0.348	0.348	0.350	0.349	0.370
49	D3	1.371	1.509	0.524	10	0.421	0.343	0.344	0.343	0.343	0.344	0.344	0.395
50	D4	1.762	1.224	0.410	47	0.401	0.339	0.344	0.339	0.339	0.340	0.340	0.352
51	D5	2.015	0.976	0.326	51	0.386	0.334	0.336	0.334	0.334	0.336	0.337	0.374

Contd...

Table 5: Contd...

Item	Correlation coefficient results												Overall desirability	
	comp. 23	comp. 27	comp. 28	comp. 30	comp. 31	comp. 33	comp. 34	comp. 38	comp. 43	comp. 47	*ri*	Rank (*ri*)	OD	Rank (OD)
1	0.981	0.988	1.000	0.820	0.734	0.774	0.984	0.871	0.965	0.971	0.909	2	0.712	1
2	0.898	0.696	0.710	0.949	0.888	0.805	0.701	0.957	0.690	0.699	0.743	12	0.622	12
3	0.572	0.568	0.574	0.627	0.705	0.630	0.562	0.636	0.562	0.578	0.603	21	0.564	20
4	0.591	0.560	0.577	0.735	0.693	0.766	0.569	0.771	0.550	0.568	0.624	20	0.544	23
5	0.565	0.548	0.542	0.608	0.724	0.622	0.550	0.575	0.552	0.551	0.590	22	0.591	15
6	0.722	0.530	0.530	0.607	0.580	0.727	0.532	0.624	0.535	0.529	0.581	23	0.530	25
7	0.531	0.523	0.529	0.591	0.606	0.653	0.537	0.590	0.517	0.529	0.559	24	0.527	26
8	0.533	0.518	0.519	0.621	0.640	0.683	0.532	0.596	0.528	0.521	0.558	25	0.568	19
9	0.509	0.504	0.512	0.562	0.662	0.591	0.511	0.597	0.497	0.508	0.539	26	0.507	28
10	0.948	0.963	0.930	0.902	0.727	0.872	0.997	0.904	0.930	0.958	0.913	1	0.689	2
11	0.948	0.938	0.974	0.804	0.978	0.592	0.897	0.861	0.897	0.991	0.889	3	0.671	3
12	0.922	0.889	0.931	0.926	0.814	0.727	0.951	0.750	0.867	0.914	0.860	4	0.656	7
13	0.911	0.858	0.884	0.786	0.850	0.755	0.882	0.845	0.839	0.912	0.845	5	0.663	5
14	0.881	0.846	0.888	0.889	0.795	0.736	0.812	0.965	0.812	0.817	0.820	7	0.632	11
15	0.878	0.804	0.827	0.977	0.981	0.691	0.880	0.965	0.787	0.800	0.826	6	0.646	9
16	0.788	0.780	0.798	0.916	0.984	0.962	0.807	0.950	0.763	0.840	0.818	8	0.656	8
17	0.765	0.765	0.776	0.962	0.960	0.951	0.776	0.948	0.763	0.796	0.807	9	0.670	4
18	0.770	0.745	0.731	0.852	0.883	0.886	0.734	0.879	0.741	0.742	0.758	10	0.660	6
19	0.738	0.707	0.701	0.806	0.762	0.812	0.713	0.723	0.701	0.720	0.743	11	0.641	10
20	0.679	0.674	0.682	0.839	0.812	0.845	0.679	0.900	0.664	0.727	0.733	13	0.604	13
21	0.669	0.666	0.657	0.751	0.885	0.753	0.690	0.792	0.647	0.683	0.705	16	0.597	14
22	0.868	0.645	0.670	0.897	0.846	0.825	0.654	0.797	0.631	0.640	0.710	14	0.586	16
23	0.676	0.630	0.647	0.976	0.839	0.965	0.625	0.965	0.616	0.645	0.708	15	0.579	17
24	0.823	0.610	0.619	0.707	0.688	0.922	0.620	0.742	0.602	0.615	0.665	17	0.563	21
25	0.603	0.598	0.608	0.703	0.784	0.755	0.604	0.805	0.588	0.622	0.644	18	0.548	22
26	0.604	0.583	0.589	0.793	0.763	0.730	0.596	0.654	0.575	0.592	0.632	19	0.570	18
27	0.536	0.494	0.502	0.548	0.645	0.601	0.487	0.656	0.504	0.503	0.539	27	0.538	24
28	0.451	0.397	0.402	0.535	0.448	0.469	0.397	0.529	0.397	0.405	0.431	39	0.441	42
29	0.417	0.392	0.397	0.430	0.463	0.438	0.393	0.540	0.401	0.407	0.420	40	0.450	39
30	0.407	0.389	0.385	0.427	0.472	0.454	0.389	0.513	0.391	0.391	0.412	41	0.449	41
31	0.397	0.381	0.388	0.411	0.458	0.436	0.385	0.543	0.386	0.403	0.408	42	0.426	44
32	0.391	0.376	0.382	0.404	0.478	0.438	0.373	0.490	0.385	0.385	0.400	43	0.452	38
33	0.508	0.480	0.489	0.586	0.581	0.610	0.488	0.642	0.489	0.509	0.523	28	0.511	27
34	0.482	0.469	0.470	0.485	0.617	0.526	0.478	0.744	0.516	0.482	0.510	29	0.499	29
35	0.473	0.461	0.468	0.517	0.562	0.527	0.467	0.763	0.475	0.487	0.502	30	0.470	31
36	0.693	0.455	0.460	0.456	0.505	0.482	0.457	0.619	0.461	0.472	0.489	31	0.470	32
37	0.638	0.446	0.452	0.557	0.486	0.580	0.450	0.549	0.451	0.473	0.486	32	0.495	30
38	0.470	0.449	0.437	0.483	0.504	0.546	0.450	0.673	0.447	0.471	0.474	33	0.455	35
39	0.453	0.433	0.441	0.520	0.502	0.500	0.438	0.654	0.439	0.461	0.465	34	0.450	40
40	0.442	0.425	0.433	0.461	0.488	0.496	0.431	0.436	0.434	0.459	0.443	38	0.467	33
41	0.438	0.416	0.416	0.454	0.462	0.490	0.427	0.607	0.422	0.452	0.443	37	0.423	45
42	0.426	0.409	0.409	0.516	0.457	0.444	0.420	0.715	0.409	0.442	0.443	36	0.408	46
43	0.710	0.403	0.409	0.506	0.417	0.544	0.410	0.509	0.403	0.417	0.444	35	0.455	36
44	0.378	0.368	0.368	0.391	0.405	0.429	0.380	0.518	0.371	0.371	0.391	45	0.402	47
45	0.364	0.363	0.363	0.375	0.384	0.404	0.363	0.429	0.363	0.410	0.378	46	0.388	49
46	0.359	0.358	0.358	0.359	0.371	0.386	0.358	0.393	0.359	0.396	0.368	48	0.389	48
47	0.385	0.353	0.353	0.397	0.467	0.424	0.355	0.436	0.357	0.369	0.394	44	0.461	34
48	0.379	0.348	0.355	0.353	0.379	0.482	0.352	0.371	0.369	0.352	0.372	47	0.454	37

Contd...

Table 5: Contd...

Item	Correlation coefficient results									Overall desirability	
	comp. 23	comp. 27	comp. 28	comp. 30	comp. 31	comp. 33	comp. 34	comp. 38	comp. 43	comp. 47	*ri*	Rank (*ri*)	OD	Rank (OD)
49	0.348	0.343	0.345	0.345	0.369	0.369	0.348	0.373	0.345	0.351	0.356	51	0.440	43
50	0.352	0.339	0.348	0.354	0.407	0.392	0.339	0.477	0.339	0.348	0.361	49	0.385	50
51	0.342	0.334	0.334	0.366	0.379	0.334	0.334	0.610	0.334	0.337	0.360	50	0.343	51

Province of China, the ethanol-soluble extraction content (T, referred to as AEC in this paper) and the total content of 2-(2-phenylethyl) chromone and 2-[2-(4-methoxy) phenylethyl] chromone of agarwood can be divided into five grades (Super grade: T ≥30.0%, total chromone content ≥1.0%. First grade: T ≥30.0%, 1% > total chromone content >0.1% or 30.0 >T ≥20.0%, total chromone content ≥1.0%. Second grade: 30.0% > T ≥20.0%, total chromone content <0.1%. Third grade: 20.0% >T ≥10.0%. Fourth grade: 10.0% >T ≥4.0%).[37] Based on the Group Standard of Zhongshan City (China), agarwood was divided into three grades by AEC (Super grade: T >20.0%, First Grade: 20.0% >T ≥15.0%, Qualified: 15% >T ≥10.0%).[38] In the previous work of this group, 98 batches of agarwood were analyzed by GC-MS. According to the AEC, agarwood samples were divided into three grades (First grade: T≥30.0%, Second grade: 30.0% > T ≥20.0%, Third grade: 20.0% > T≥10.0%).[39] In this study, there were significant variations in the AEC of all samples, ranging from 5.0% to 36.1%. Comprehensive analyses of the various species, different inducement methods, and the AEC were performed [Figure 5a]. Using the same FAM71 inducer, the AEC of *A. malaccensis* was higher than *A. sinensis*, and there was a significant correlation between the AEC and species ($P = 0.007$ ($P < 0.01$)). A comparison of different inducers revealed that the AEC of *A. sinensis* was the highest when induced by FA, followed by FAM71, and finally by NA8. However, there was no significant difference in AEC between the three different induction methods.

The AEC did not increase even with a longer resin production time. Therefore, analyses of the resin formation time and AEC were carried out, as well as fitting of the relevant curves. The data were presented as an average ± SEM [Figure 5b]. The results showed that the longer the resin formation time, the higher the AEC 11 months ago. At 12 months, the AEC was significantly lower than at 11 months, with AEC declining after the 11th month. The average value of AEC was at its lowest in the 24th month. There may also have been a reduction during the resin production process; a longer resin formation time did not necessarily mean, a higher AEC value. Therefore, the 11th month is recommended as the best time to collect based on the analysis of dynamic changes in AEC and resin formation time.

In the correlation data analysis, there was a significant correlation between agarotetrol and AEC ($P = 0.000$ ($P < 0.01$)). Agarotetrol is one of the THPECs commonly found in agarwood. However, because PECs in chromone compounds can only be detected using GC-MS, agarotetrol was determined using the HPLC method. Using the same FAM71 inducer, the agarwood samples were compared according to *A. malaccensis* (A1–A26) and *A. sinensis* (B1–B24), respectively. It was discovered that agarotetrol significantly correlated with AEC ($P < 0.01$) [Figure 5c]. However, C1–C3 and D1–D5 had no correlation with AEC, which could be due to the limited number of samples. In addition, it was possible that there was no correlation between agarotetrol and AEC under FA or fungal-induced conditions. Therefore, further investigations are still required.

CONCLUSION

In this paper, the gray correlation degree and TOPSIS analysis were used to comprehensively analyze the 18 characters, including AEC, the content of agarotetrol, R, and 15 secondary metabolites representing 58 batches of samples. It was demonstrated that the quality of agarwood from *A. malaccensis* induced by FAM71 was better than that of *A. sinensis*. Furthermore, the quality of agarwood induced by FAM71 was also better than that induced by FA or NA8 alone. These findings provided a theoretical basis for the selection of high-quality agarwood inducer and tree species, as well as a reference basis for efficient production of agarwood in the actual production process.

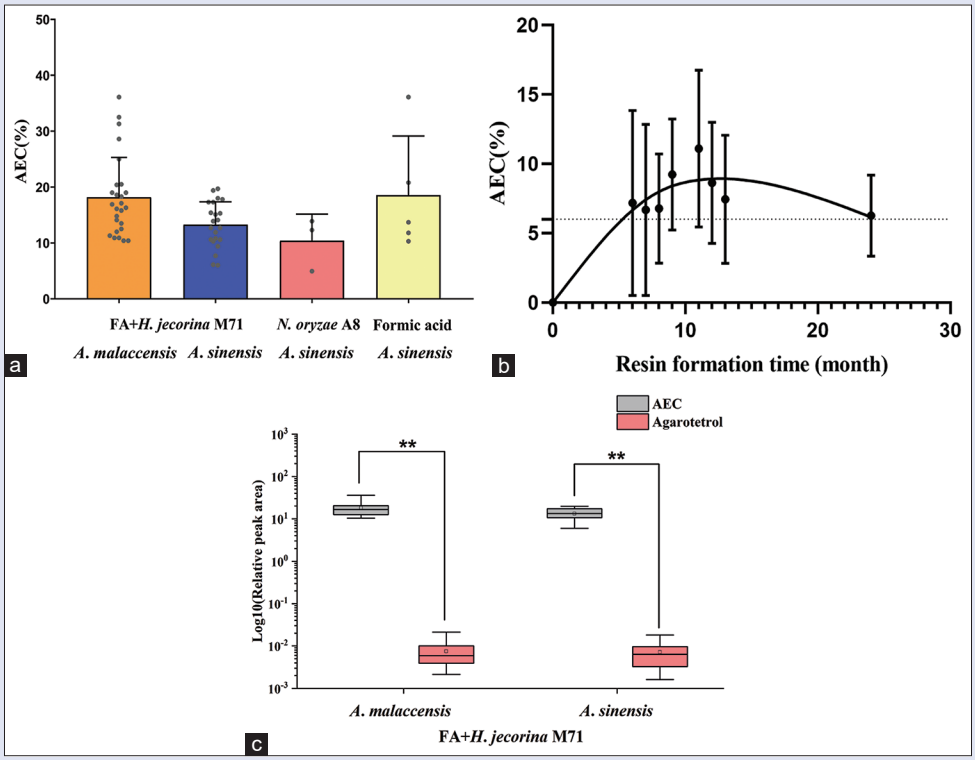

Figure 5: The AEC of all samples (a). The correlation curve between the resin formation time and AEC of the agarwood of FAM71 (b). Box plot of the content of agarotetrol and AEC in *A. malaccensis* and *A. sinensis* (**$P < 0.01$) (c)

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Author contributions

Xiaoxia Gao and Weimin Zhang conceived and designed the experiments; Weimin Zhang contributed reagents and materials; Shimin Deng, Pengjian Zhu, Xin Zhou, and Xiaoying Chen performed the experiments; Zhiling Zhuang and Shenghong Wu analyzed the data; and Zhiling Zhuang wrote the paper.

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Conflicts of interest

There are no conflicts of interest.

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