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Original Article

Temporal characteristics of agarwood formation in *Aquilaria sinensis* after applying whole-tree agarwood-inducing technique

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ABSTRACT

Objective: Agarwood—a resinous wood produced by *Aquilaria* plants in response to injury or artificial induction—is a valuable medicinal and fragrance resource. Whole-Tree Agarwood-Inducing Technique (Agar-WIT) has been widely used to produce agarwood. However, the time-dependent characteristics of agarwood formation induced by Agar-WIT are yet to be clarified. To promote technologically efficient utilization and upgradation of Agar-WIT, the dynamic process and mechanism of agarwood formation were analyzed for one year.

Methods: Agarwood formation percentage, barrier layer microscopic properties, extract levels, compound level, and characteristic chromatograms of agarwood were examined by referring to the *Chinese Pharmacopeia* (2020 version).

Results: Agar-WIT could maintain a high percentage of agarwood formation over one year compared with that of healthy plants. Alcohol-soluble extract and agarotetrol levels showed fluctuating cyclic changes with peaks occurring first during the fifth and sixth months, and subsequently in the 11th month. *Aquilaria* trees subjected to Agar-WIT treatment for 1–12 months showed significant characteristics of a dynamic agarwood formation process. The barrier layer began to appear in the fourth month after treatment. Alcohol-soluble extractive levels in agarwood formed in the second month, and thereafter, exceeded 10.0%, and agarotetrol in agarwood produced after four months or later, exceeded 0.10%.

Conclusion: According to the *Chinese Pharmacopoeia*, alcohol-soluble extractive levels in agarwood should not be less than 10.0% and agarotetrol level should exceed 0.10%. After four months of Agar-WIT treatment, the formed agarwood theoretically met these standards and was suitable for developed and utilization. However, the optimal harvest time was found to be the 11th month, followed by the sixth month after Agar-WIT treatment. Therefore, Agar-WIT resulted in swift agarwood formation and stable accumulation of alcohol-soluble extracts and agarotetrol. Thus, this method is efficient for large-scale cultivation of *Aquilaria sinensis* to produce agarwood and provide raw materials for the agarwood medicinal industry.

1. Introduction

The genus *Aquilaria* comprises valuable, non-timber tree species known for their ability to form agarwood after sustaining some form of injury or infection (Azren, Lee, Emang, & Mohamed, 2019; Liu et al., 2013). Agarwood has long been used and traded in several Asian countries, the Middle East, Europe, and the United States. As one of the most famous traditional medicines in China, agarwood plays an important role in the management of ailments requiring sedative, carminative, and antiemetic treatment (Wang et al., 2018). It has been confirmed that the medicinal properties of agarwood extract are attributable to its principal compound chromone derivatives and sesquiterpenoids (Pan, Qiu, He, Li, & Shen, 2019). Moreover, agarwood was traditionally used as a spice and aromatic agent in several spiritual practices (Hashim, Kerr, Abbas, & Salleh, 2016; López-Sampson & Page, 2018). However, the natural formation process of agarwood is time-consuming, and excessive commercial demand has resulted in its overexploitation. Owing to the difficulties in plant regeneration under adverse

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human influence, several natural *Aquilaria* populations are on the verge of extinction (Chen, Liu, & Heinenm, 2019). All species of *Aquilaria* and *Gyrinops* appear in the Appendix II list of *the Convention on International Trade in Endangered Species of Wild Fauna and Flora*, since 2004 (Amendments to appendices I and II of CITES, 2004).

Several Asian countries have been planting *Aquilaria* trees on a large scale to protect the endangered wild species and provide sufficient agarwood resources to meet global demand (Azren et al., 2019). In China, there are two species of *Aquilaria*, one is *A. sinensis* (Lour.) Gilg and the other is *A. yunnanensis* S. C. Huang. The wild *A. sinensis* is distributed in Hainan, Guangdong, Guangxi, and Yunnan Provinces, whereas *A. yunnanensis* is only distributed in the border of Yunnan Province. The cultivated *A. sinensis* is grown on a large scale in Hainan, Guangdong, Guangxi, Yunnan and Fujian provinces, as its wild resource is shrinking. Preliminary estimates indicate that there are more than 50 million *A. sinensis* trees suitable for producing incense.

Owing to the narrow distribution area of *A. yunnanensis*, most of the agarwood produced in China is from *A. sinensis*. For thousands of years, agarwood in China has been procured from wild resources. However, because of disordered logging in recent decades, *A. sinensis* has been listed as a national second-class protected plant. Currently, almost all agarwood is obtained from cultivated *A. sinensis* resources. In order to make the tree produce agarwood, various methods have been devised. Existing traditional production techniques include partly-trunk-pruning, burning-chisel-drilling, drilling, cutting, and nailing (Kadir, Azizan, & Othman, 2020; Wu, Liu, Li, Yu, & Lin, 2020), which can produce agarwood but cannot meet large-scale supply. Conversely, modern production techniques include cultivated agarwood kits (CA-Kits) (Blanchette & Van Beek, 2005), the fungi-inoculation method (Chen et al., 2018), and the whole-tree agarwood-inducing technique (Agar-WIT), which is one of the most widely used artificial agarwood-induction methods in China and other countries (Yang et al., 2019). Development of this technique was based on the theory of plant defense response invoked in *Aquilaria* trees upon being wounded (Zhang et al., 2010). A transfusion device is used to inject efficient and safe inducers into the trunk; the inducers are then transported to all parts of the plant via its water transportation system, causing internal wounds and resulting in the formation of agarwood, after a certain period of accumulation under stimulation (Liu et al., 2013). Compared with conventional and other non-conventional agarwood production methods, Agar-WIT offers the advantages of high and stable yield, quality, and efficiency, as well as a product that is suitable for use as pharmaceutical raw material (Zhang et al., 2010). Agar-WIT has been extensively and successfully applied to different species in several countries (Yang et al., 2019). This technique has also been the basis of upgraded techniques and many derivative methods have been developed or are under development.

Agarwood is the product of the plant's defense against external damage (Huang et al., 2013), and its formation leads to changes in xylem structure (Zhang, 2013). After injury induction, the xylem structure appears evidently stratified, and distinct decay, agarwood, transitional, and healthy layers can be identified, based on color and morphological characteristics (Liu et al., 2019). Resin is found in the interxylary phloem–a prominent structural feature of *Aquilaria* (He, Pan, Liang, Luo, & Qiu, 2019) and ray cells of the agarwood and transitional layers (Zhang, 2013). In addition to the ability to produce resin, the interxylary phloem can also form a barrier layer to prevent thickening of the agarwood layer (He et al., 2019). The main components of agarwood are sesquiterpenes and chromone compounds (Faizal et al., 2022; Ma et al., 2019; Nasution, Siregar, Miftahudin, & Turjaman, 2020), of which 2-(2-phenylethyl)chromone is characteristically present.

The agarotetrol–5,6,7,8-tetrahydroxy-2-(2-phenylethyl)chromone—level reflects the quality of agarwood to a certain extent (Chhipa & Kaushik, 2020; Lancaster & Espinoza, 2012). The level of alcohol-soluble extract in agarwood is also an important indicator for evaluating its quality; higher extract levels typically equate to agarwood of better quality (Liu, Wei, Gao, Zhang, & Lyu, 2017). Moreover, there is a certain correlation between the alcohol-soluble extract level and the representative sesquiterpene constituents such as lignocellulosic acid and aromatic benzyl acetone (Zhou et al., 2016).

The structure of the barrier layer and the levels of agarotetrol and alcohol-soluble extract are important factors affecting the yield and quality of agarwood. However, only a few studies have been conducted on the temporal characteristics of agarwood. Furthermore, the ideal time to harvest agarwood has not been systematically studied. In this study, the dynamic process of agarwood formation by means of Agar-WIT was monitored and the percentage of agarwood, barrier layer, alcohol-soluble extract, and agarotetrol level were analysed, over a 12-month period. To the best of our knowledge, this is one of the few studies to comprehensive analyze the dynamic formation of agarwood via Agar-WIT. Our study will aid in guiding more efficient production and harvest of agarwood, while providing a scientific reference for the efficient utilization and technical upgrade of Agar-WIT.

2. Materials and methods

2.1. Plant materials

The experiment was conducted in Xinglong Town, Wanning City, Hainan Province, China (18°39′–18°41′N, 110°27′–110°31′E). The Agar-WIT, invented by our group (Wei, Yang, Zhang, Meng, Feng, & Gan, 2010), was used to stimulate agarwood formation in 39 three-year-old *A. sinensis* trees of similar trunk girth. The liquid delivered by each tree was 100 mL, and each tree was inoculated once. From the first month after treatment, agarwood samples of three trees were collected monthly for 12 consecutive months, as shown in Table 1. Healthy *A. sinensis* served as a control group. All the trees were taken back whole. The materials in this study were all from the main trunk. The results were examined against the *Chinese Pharmacopeia* (2020 version) standards. All treatments in this study were repeated with three trees. Samples were identified by Rongtao Li, associate researcher from Hainan Branch Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences. Voucher specimens have been maintained in the Herbarium of the Agarwood Appraisal Centre, Hainan Branch Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences.

Table 1
Information on agarwood samples.

Sample codes	Treatment time (month)	Treatment date	Harvest date
S0	0	2018.01.01	2018.01.01
S1	1	2017.12.01	2018.01.01
S2	2	2017.12.01	2018.02.01
S3	3	2017.12.01	2018.03.01
S4	4	2017.12.01	2018.04.01
S5	5	2017.12.01	2018.05.01
S6	6	2017.12.01	2018.06.01
S7	7	2017.12.01	2018.07.01
S8	8	2017.12.01	2018.08.01
S9	9	2017.12.01	2018.09.01
S10	10	2017.12.01	2018.10.01
S11	11	2017.12.01	2018.11.01
S12	12	2017.12.01	2018.12.01

2.2. Agarwood yield estimation

To calculate agarwood productivity, we cut down each tree after the indicated agarwood formation period, and divided the trunk into three sections. The upward cross-section of each short trunk portion was imaged using a digital camera. We then calculated the number of pixels in the total cross-sectional (N_C) and agarwood layer areas (N_A) of each tree, using Photoshop software (Chen, Zhou, Pan, Wang, & Zeng, 2010). The proportional areas of the agarwood layer (S_A) and total cross-sectional surface (S_C) could equate to the number of agarwood and cross-sectional layer pixels, respectively. We assumed that the trunk was a standard cylinder, and the ratio of resin area to the total area of each tree was constant along the length of the trunk. The agarwood area ratio of each tree was calculated using the following formula:

$$\frac{S_A}{S_C} = \frac{N_A}{N_C}$$

2.3. Anatomical transformation observation

Divide the trunk into three sections. The upward cross-section of each short trunk portion was material for anatomical transformation observation. We cut the agarwood samples into small square pieces (0.5 cm × 0.5 cm) and soaked them in 70 °C water for 6 h, until soft. Thereafter, the pieces were cut into 50-μm slices using a cryostat (CM 1950, Leica Biosystems, Weztlar, Germany). Finally, the slices were placed in chloral hydrate solution (Macklin Biochemical Co., ltd., Shanghai, China) for 5 h until transparent and the cross-sectional structure of each sample was observed under a microscope (Eclipse 80i, Nikon, Tokyo, Japan).

2.4. Alcohol-soluble extract level determination

Alcohol-soluble extract level was determined according to the method described in the Chinese Pharmacopoeia (Editorial Board of the Chinese Pharmacopoeia Commission, 2020). Briefly, 2 g of powdered agarwood sample was immersed in 50 mL of 95% ethanol in an Erlenmeyer flask and weighed. After being left to stand for 1 h, the flask was connected to a reflux condenser tube, and heated until the solution boiled slightly in the Erlenmeyer flask, allowing reflux for 1 h. After cooling, the Erlenmeyer flask with sample level was weighed again, 95% ethanol was used to make up for the lost weight, and the mixture filtered through dry filter paper. The filtrate (25 mL) was measured and placed in a draught drying cabinet (DHG-9053A; Tuopu Instrument Co., ltd., Lianyungang China) and dried at 105 °C for 3 h to a constant weight and dryness, before being cooled in a dryer for 30 min. The weight was immediately and accurately measured. The level of alcohol-soluble extract in each sample was calculated using the dry product, and each experiment was repeated twice.

2.5. Thin-layer chromatography

Agarwood samples (121222–201203) were purchased from the National Institutes for Food and Drug Control (Beijing, China), as standard product control samples. Agarwood powder (0.5 g of each), control and experimental sample, was combined with 30 mL of ether (Xilong Scientific Co., ltd., Shanghai, China) for 1 h, and the mixture was subjected to ultrasonic extraction (40 kHz, 250 W) using an ultrasonic cleaner (SB25-12DTDN; Ningbo Scientz Biotechnology Co., ltd., Ningbo China). The extract was filtered and dried, before adding 2 mL of trichloromethane (Xilong Scientific Co., ltd., Shantou, China) to dissolve the extract, for use as the test solution. To conduct thin-layer chromatography (TLC), 10 μL of the solution was transferred by capillary tube onto a 10 cm × 20 cm, GF254 TLC Silica gel plate (7631–86-9; Merck, Burlington, MA, USA), with chloroform:diethyl ether (10:1) as the mobile phase, and examined under a ultraviolet (UV) lamp (JY02S, Junyi Electrophoresis Co., ltd., Beijing, China) at wavelengths of 365 nm. A total of 39 samples were analyzed.

2.6. Characteristic chromatogram identification and agarotetrol level determination using high-performance liquid chromatography

Agarotetrol (111980–201601) and standard agarwood samples were bought from the National Institutes for Food and Drug Control in China. Standard (control) and experimental agarwood powder (0.2 g of each) was mixed with 10 mL of 95% ethanol and subjected to ultrasonic water bath extraction for 1 h. The extract solution was cooled to room temperature and weighed; the lost weight was made up with 95% aqueous ethanol. The solution was then filtered through a 0.45-μm membrane filter before injection into the high-performance liquid chromatography (HPLC) system (2695–2475; Waters Technologies ltd., Shanghai, China). The agarotetrol standard sample was dissolved in ethanol at 60 μg/mL. Moreover, the chromatographic condition was aligned to the Chinese Pharmacopoeia guidelines.

3. Results

3.1. Macroscopic characteristics of agarwood

Cross-sections of *A. sinensis* following Agar-WIT treatment for one year were shown in Fig. 1. The trunk cross-section was divided into four layers, namely, from the outside to inward, the healthy layer, transition layer, agarwood layer, and decay layer. A significant agarwood layer had formed within one month after induction (Fig. 1A). The most prominent macroscopic change in the agarwood layer during the one-year experimental period was that the outer contour deepened in color, as represented by the change from a dashed brown line to a solid black line in Fig. 1E–L, starting from the fifth month. However, the area of the agarwood layer did not expand unhindered over time. The barrier layer began to appear in the fourth month after treatment, and its structure was significantly established in the sixth month (Fig. 1D–L). In contrast, the untreated plants did not have a stratified structure in the cross section (Fig. 1M).

3.2. Percentage of agarwood

In this study, the representative percentage of agarwood, as an indicator of agarwood yield, refers to the ratio of the resin area to the total cross-sectional area. Overall, the agarwood yield of healthy plants is zero. Whereas, the agarwood percentage obtained by Agar-WIT showed an apparent monthly variation but a generally ascending trend over 12 months (Fig. 2). One month after the induction treatment, the agarwood percentage reached 10.33%; peaked at 18.82% and 18.76% during the fourth and ninth months, respectively; following which, it continued to decrease up to the end of one year.

3.3. Microscopic characteristics of agarwood

Based on microscopic analysis, the cross-sections of all samples displayed interxylary phloem, xylem ray, vessel, and fiber structures. The interxylary phloem was an elliptical or elongated strip intersected with xylem rays, which contained 1–2 columnar cells, whereas the vessel as a rounded polygon. The interxylary phloem, rays, and some vessels were filled with resin, and the color of the resin changed from yellowish-brown to darker brown as treatment

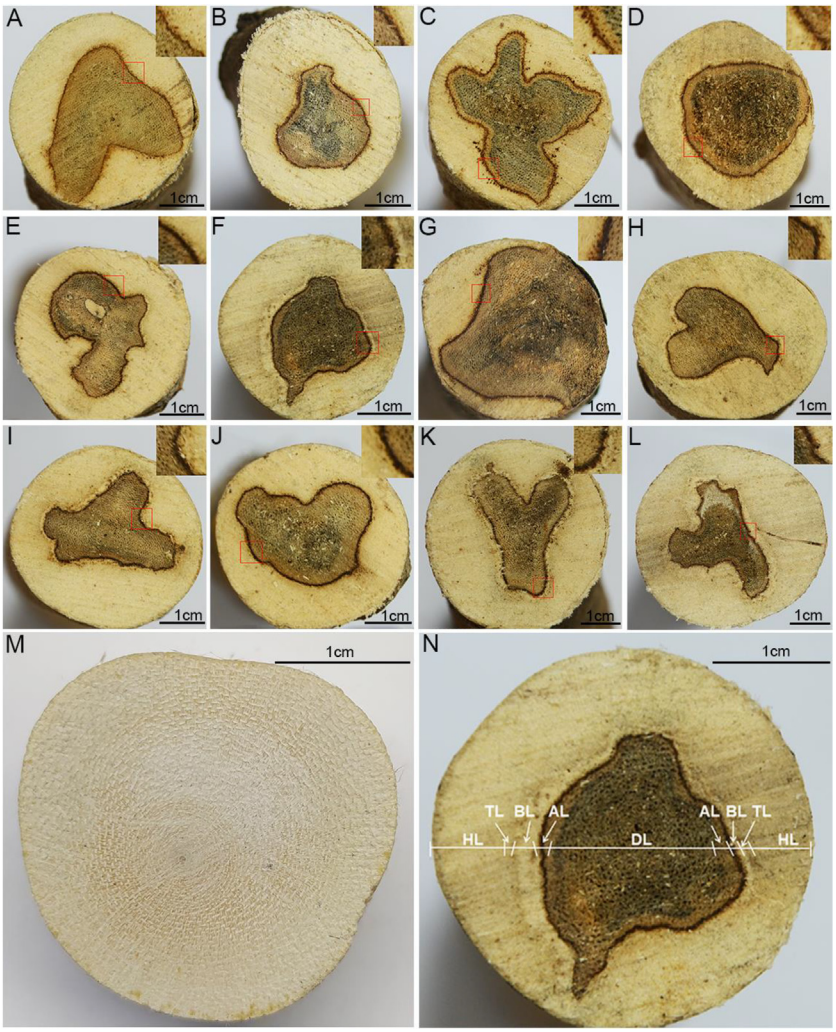

Fig. 1. Macroscopic transformation of agarwood produced by Agar-WIT during a 12-month experimental period. A–L represent the trunk cross-sections of *A. sinensis* trees harvested once per month for each of 12 months of the experiment; M, cross-section of a healthy plant; N, *A. sinensis* stem structure after Agar-WIT treatment (DL, decay layer; AL, agarwood layer; BL, barrier layer; TL, transitional layer; HL, healthy layer).

duration progressed (Fig. 3A–L), but in healthy plants, the structures were clean and unfilled (Fig. 3h1-h2). By two months after treatment, a part of the interxylary phloem had enlarged abnormally (Fig. 3N) and fused to form a barrier layer, by the fourth month. This pattern was consistent with the macroscopic finding that the formation of the barrier layer cleaved the resin-containing interxylary phloem and ray structures into two parts and prevented them from extending further (Fig. 3P–X).

3.4. Identification of agarwood using thin-layer chromatography

TLC chromatogram results were shown in Fig. 4. At UV wavelengths of 365 nm the experimental agarwood samples displayed spot patterns similar to the standard agarwood samples. Compared with the agarwood produced using Agar-WIT and standard agarwood, only a fuzzy point could be found in the healthy plant samples. The results confirmed that the resins obtained each month for one year following Agar-WIT treatment were classified as agarwood.

3.5. Alcohol-xoluble extract and zgarotetrol levels

In healthy plants, less alcohol-soluble extract and almost no agarotetrol were detected. However, the levels of the two components in the samples produced using the Agar-WIT were significantly increased. The levels of alcohol-soluble extract and

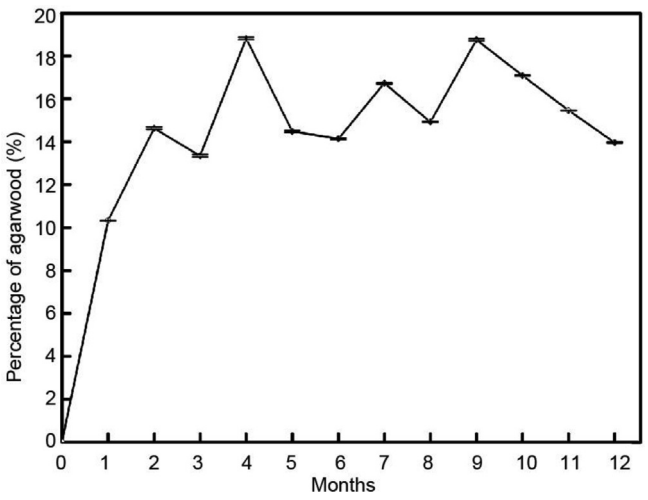

Fig. 2. Percentage of agarwood produced by Agar-WIT during a 12-month experimental period. 0 represents the percentage of agarwood in healthy plant samples; 1–12 represent the agarwood percentage obtained by Agar-WIT.

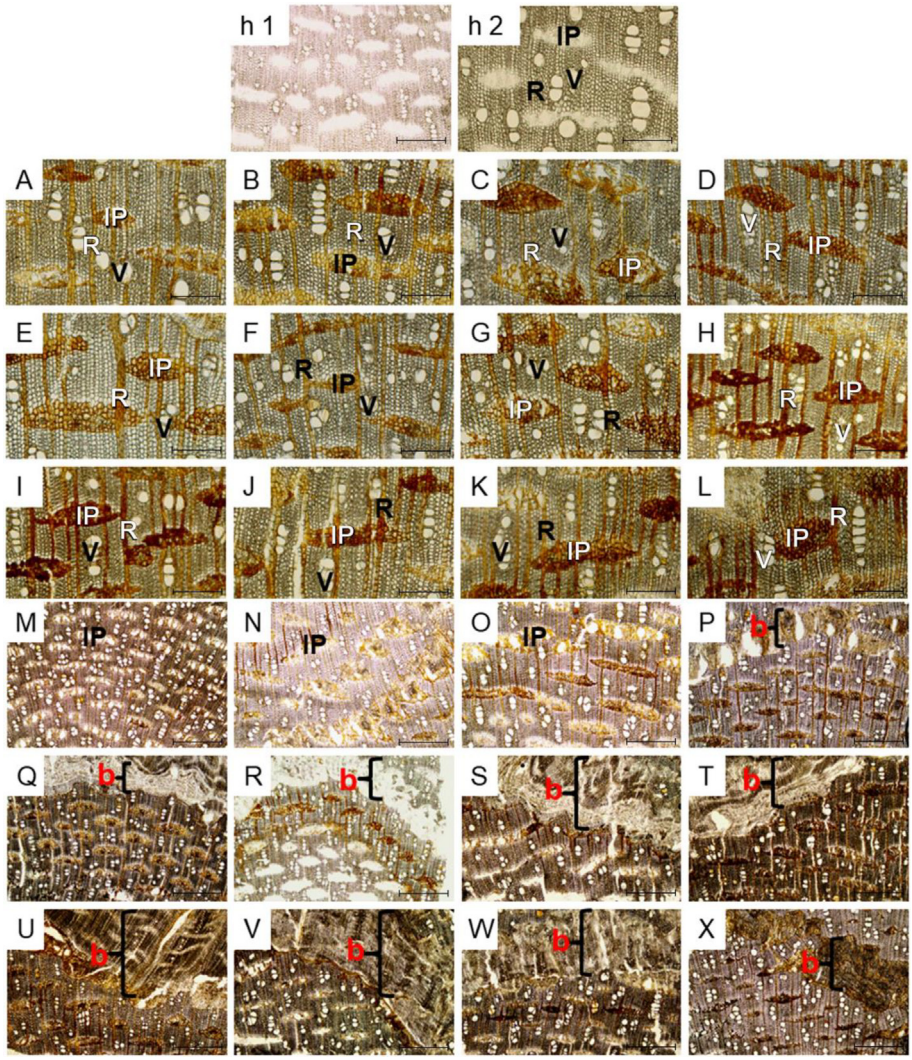

Fig. 3. Anatomical transformation of agarwood produced by Agar-WIT during a 12-month period. h1–h2 represent anatomical characteristics of healthy plants. A–L respectively represent the monthly anatomical changes in agarwood over 12-month period; M–X respectively represent the monthly anatomical changes in the barrier layer over the 12-month period. IP, Interxylary phloem; V, vessel; R, xylem ray; b, barrier layer.

agarotetrol in agarwood produced after Agar-WIT treatment over a 1–12 month range showed fluctuating cyclic patterns (Fig. 5), with three distinct periods over the 12 months. Specifically, the levels increased rapidly in the first six months, decreased slightly from the sixth to 10th month, and rebounded sharply in the last two months. Both alcohol-soluble extract and agarotetrol levels peaked first in the fifth or sixth month, and then in the 11th month. It is worth noting that the alcohol-soluble extract level in agarwood was consistently above 10.0% from the second month. Additionally, the agarotetrol level in agarwood was consistently above 0.10% from the fourth month. A positive correlation was observed between the levels of agarotetrol and alcohol-soluble extract.

3.6. High-performance liquid chromatography characteristic chromatograms

The agarwood samples produced using Agar-WIT were analyzed using HPLC; the samples (S1–S12) showed six characteristic peaks (Fig. 6), which were consistent with the characteristics of standard agarwood (CK). In contrast, samples from healthy plants (S0) showed no significant peaks. Based on the Chinese Pharmacopoeia (2020 edition) guidelines, peak 1 was identified as agarotetrol, peak 3 as 8-chloro-2-(2-phenylethyl)-5,6,7-trihydroxy-5,6,7,8-tetrahydrochromone, and peak 5 as 6,4′-dihydroxy-3′-methoxy-2-(2-phenylethyl)chromone. The characteristics of agarwood produced after Agar-WIT treatment for 1–12 months, as observed in our study, aligned with those of the standards set in the *Chinese Pharmacopoeia* (2020 edition).

4. Discussion

4.1. Changes in percentage and structure of agarwood during formation

Agarwood accumulates to resist damage inflicted to a plant. In this study, stimulation by Agar-WIT caused the xylem of *A. sinensis* stem to change horizontally from the inside out, with the formation of a decay layer, agarwood layer, transitional layer, and healthy layer; moreover, the agarwood layer formed in a vertically continuous sheet-like configuration. After a period of stimulation via Agar-WIT, a white band-like barrier layer structure formed between the agarwood and transitional layers, because of the dif-

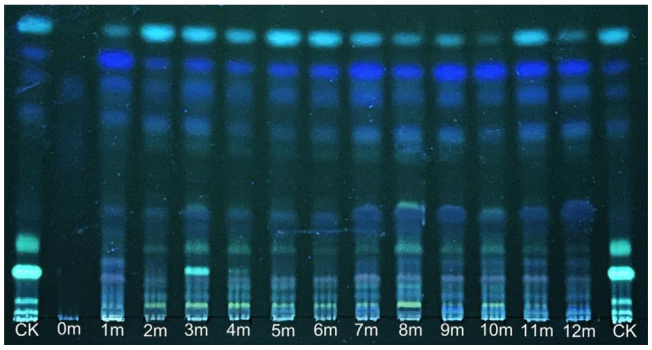

Fig. 4. TLC Chromatogram of agarwood produced using Agar-WIT during a 12-month period. 0 m represents for the healthy plant samples; 1 m–12 m represent the agarwood produced using Agar-WIT; CK represents the standard agarwood. TLC was conducted at a UV wavelength of 365 nm.

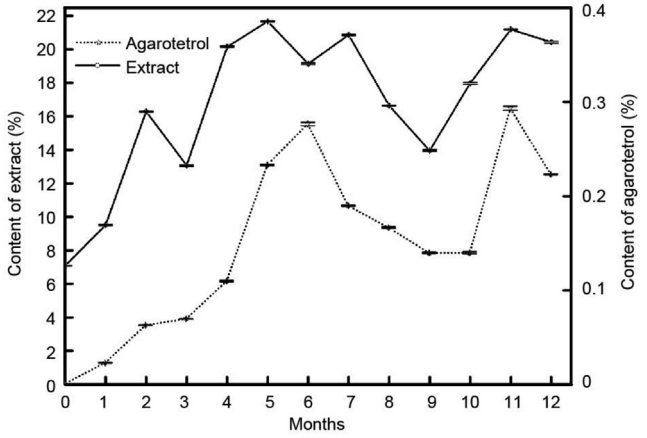

Fig. 5. Alcohol-soluble extractive and agarotetrol levels in agarwood produced using Agar-WIT, during each month of a 12-month period. 0 represents the alcohol-soluble extractive and agarotetrol levels in healthy plant samples; 1–12 represent the alcohol-soluble extract and agarotetrol levels in agarwood produced after Agar-WIT treatment.

ferentiation of parenchyma cells in the interxylary phloem adjacent to the agarwood layer (Zhang et al., 2012). It was found that the formation of this barrier layer restricted the extension of the resin-containing interxylary phloem and ray structures, which was consistent with previous findings that the barrier layer hinders the lateral thickening of the agarwood layer (He et al., 2019; Liu, Yang, Zhang, Yang, & Liu, 2016). Furthermore, the proportion of agarwood formation was higher overall and showed an upward trend over the 12-month experimental period. However, the percentage of agarwood growth fluctuated during the first nine months and decreased continuously thereafter; therefore, we speculated that the thickness of the agarwood layer would lessen to a certain extent, should the barrier layer continue to grow. Therefore, we inferred that a longer injury induction-time does not necessarily enhance agarwood formation. The barrier layer can resist external damage, together with the agarwood layer (Zhang et al., 2012), as the formation of the latter represents the plant's chemical protection mechanism, whilst barrier layer formation represents its means of physical defense (Liu et al., 2019). Elimination or reduction of the barrier layer could represent an important avenue for improving agarwood yield and should be further investigated.

4.2. Changes in chemical composition of agarwood

Agarwood is mainly formed and accumulated in the interxylary phloem, vessel, and ray cells, and its main components are sesquiterpenes and chromone compounds (He et al., 2019). The agarotetrol level in agarwood is related to its production time (Takamatsu & Ito, 2020), whereas the levels of alcohol-soluble extract may be affected by production time and oil level (Li et al., 2017). In this study, the levels of extracts and agarotetrol in agarwood produced during the experimental period showed an overall upward trend with treatment progression. Their levels increased rapidly in the first six months, which may reflect the correlation between agarwood formation and external damage, previously reported in the context of the defense response of *A. sinensis* (Gao, Wei, Yang, Zhang, & Zhao, 2012). Briefly, when inducers are injected into healthy *A. sinensis*, a large amount of resin initially accumulates to resist the damage. Thereafter, a slight decrease

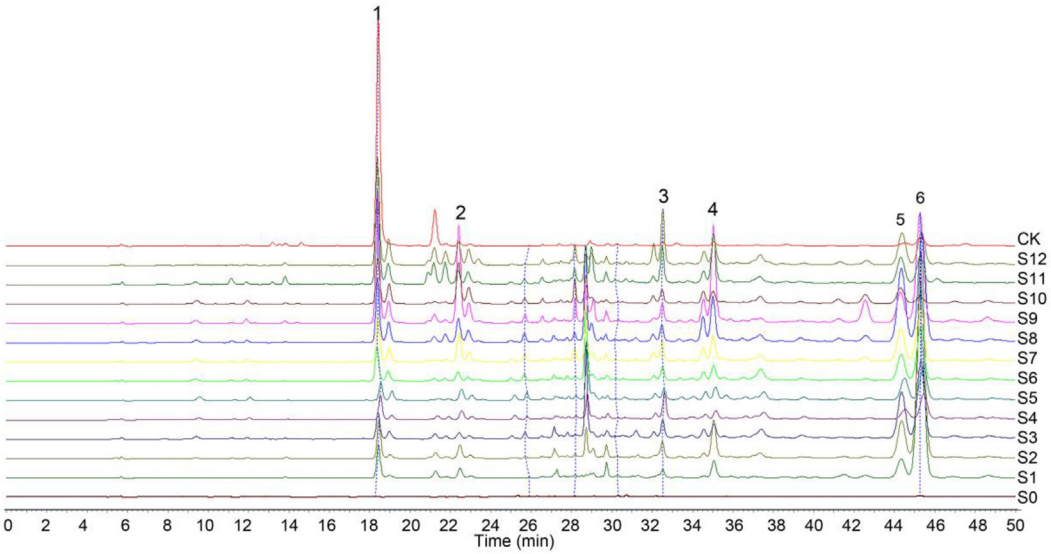

Fig. 6. HPLC characteristic chromatograms of agarwood produced using Agar-WIT during a 12-month period. S1–S12 represent the monthly HPLC characteristic chromatograms of agarwood over 12 months; S0 represent the HPLC characteristic chromatograms of healthy plant samples; CK represents the HPLC characteristic chromatograms of the standard agarwood.

may be related to the formation of the barrier layer. Moreover, a significant positive correlation was observed between the levels of agarotetrol and alcohol-soluble extract in agarwood, within the 1-year experimental period. Liu (2018) also pointed out that, besides *A. sinensis*, a positive correlation exists between the levels of agarotetrol and alcohol-soluble extract in agarwood from *A. crassna*, *A. malaccensis* and *A. yunnanensis*, subjected to Agar-WIT treatment. Therefore, based on the change trend of alcohol-soluble extract and agarotetrol, agarwood should be harvested in the period when the levels of these two substances are high.

4.3. Harvest time of agarwood produced using Agar-WIT

TLC, fingerprint profiles, the representative percentages of agarwood, alcohol-soluble extract, and agarotetrol are important indicators for a comprehensive system of evaluating agarwood quality. Based on this quality evaluation, the ideal harvest time can be inferred. In this study, all agarwood quality indicators met the standards set in the Chinese Pharmacopoeia (2020 edition), after four months. However, the levels of the three indicators did not continuously increase with the extension of Agar-WIT treatment time. Therefore, agarwood should be harvested at the correct time to achieve the best quality and yield within one year, considering the effect of barrier layer formation. Thus, based on the premise that overall agarwood yield is high when the levels of agarotetrol and alcohol-soluble extract are the highest in the sixth and eleventh months of treatment in this experiment, these time points can be used as a guide for determining the optimal harvest time within 1 year.

5. Conclusion

Our results confirmed that Agar-WIT is an efficient method for producing agarwood during large-scale cultivation of *A. sinensis*. The area ratio of agarwood produced using Agar-WIT in one year could reach 10%–18%. However, the formation of a barrier layer prevented continuous horizontal thickening of the agarwood layer. The levels of alcohol-soluble extract and agarotetrol in agarwood increased periodically, and they positively correlated. Agarwood could be successfully harvested after four months of Agar-WIT stimulation, with the ideal harvest time inferred to be around 6 and 11 months of the treatment period. Considering the shortage of wild resources and increasing demand for high-quality agarwood, this study not only provides data support for the promotion and utilization of Agar-WIT, but also has referential significance for the development of even more efficient production techniques. The agarwood layer formed in a vertically continuous sheet-like configuration by Agar-WIT, while other methods which included the scorched holing method, bark peeling method, insect larvae gnawed resulted in thin layered area, fasciculate area or punctate area (He et al., 2019). It indicates the area of agarwood formation by Agar-WIT is obviously larger. The extract and total chromone contents of agarwood produced by Agar-WIT from the six producing areas in China were higher than those from the five producing areas in Southeast Asia (Yang et al., 2019). The methods used for inducing agarwood formation affect the chemical constituents of agarwood. But agarwood formation and accumulation in *A. sinensis* is a complex process (Yan, Yang, Chen, Wang, & Li, 2019). Moreover, agarwood quality is affected by various factors, and its composition is diverse. It is still hard to conclude that those molecules are quality determinants (Yan et al., 2019). Further studies combining both chemicals analysis and medical effects or fragrant assays might help to identify the qualification markers. Therefore, the dynamic process of agarwood formation induced by Agar-WIT requires further study.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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