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Production of high-quality agarwood in *Aquilaria sinensis* trees *via* whole-tree agarwood-induction technology

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Abstract

We used whole-tree agarwood-induction technology to produce agarwood from *Aquilaria sinensis* trees within 20 months, and evaluated the quality of this agarwood. The results showed its characteristics were similar to those of high-grade wild agarwood in terms of texture, chemical constituents, essential oil content, and ethanol-soluble extract content, with the lattermost quality far surpassing the requirement of traditional Chinese medicine agarwood, as indicated in *Chinese Pharmacopoeia 2010*. To the best of our knowledge, this is first study to show that high-quality agarwood can be produced in whole *A. sinensis* trees *via* a chemically induced technology.

Keywords: Agarwood; *Aquilaria sinensis*; Chemically induced; Quality; Whole-tree agarwood-induction technology

Agarwood is the resinous wood of the *Aquilaria* tree. Neither wild nor cultivated *Aquilaria* trees can form agarwood without wounding induced by external factors such as physical injury, insect gnawing, or microbial infection. In addition, an *Aquilaria* tree takes several years to form agarwood around the wound. Agarwood plays roles in traditional Chinese medicine for its sedative, carminative, and anti-emetic effects, and also as incense for religious ceremonies [1]. Further, agarwood essential oil is the most important ingredient in high-end perfumes because of its unique fragrance. Several factors have caused the depletion of wild *Aquilaria* trees, including agarwood's immense value and rarity, indiscriminate cutting of trees, and over-harvesting. As a result, *Aquilaria* spp. have been listed as ''endangered'' in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora [2].

Great efforts have been made to preserve natural *Aquilaria* resources and increase the supply of agarwood, *e.g.*, by increasing cultivation of *Aquilaria* trees, and intentionally injuring trees to produce agarwood. To date, Indonesia,

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Cambodia, Thailand, Vietnam, China, as well as other countries, have established *Aquilaria* plantations [3]. Farmers usually use axe chopping, nailing, and holing to produce low-grade agarwood. However, it often takes a long time to produce agarwood by these methods, and the yield is generally poor [4].

An efficient alternate method patented in China by our laboratory and being international patent pended, which is called the whole-tree agarwood induction technology (Agar-Wit) [5,6], has been developed to induce the formation of high-quality agarwood in a shorter time period. A degradable chemical solution that induces agarwood production was conveniently injected into the xylem of *A. sinensis* trees through the transfusion sets. Because of the transpiration pull of water, this inducer is transported throughout the tree, leading to systemic wounds. Over a period of several months, agarwood forms around the wounds. In this study, the quality of the induced agarwood was analyzed and evaluated. To the best of our knowledge, this is the first study to show that high-quality agarwood could be produced in cultivated *A. sinensis* trees in just 20 months by using Agar-Wit.

1. Experimental

In this study, 6 agarwood samples were analyzed and compared with healthy fresh white wood (Table 1). The C1 and C2 agarwood samples were preliminarily assessed by senior collectors with extensive experience from the Agarwood Association of Hainan, China.

Various characteristics of the agarwood were then assessed by thin-layer chromatography (TLC), including ethanol-soluble extract content and essential oil content [7]. TLC silica gel 60 F_{254} glass plates were purchased from Merck. The main compounds in the essential oil were identified using GC–MS or GC–RI, which was mature method and developed by Huaiqiong Chen in our laboratory. The stability and reproducibility of the method has been tested in her manuscript [8].

2. Results and discussion

The agarwood induced *via* Agar-Wit was of high quality, and was further classified as *tagara* by collectors. With ideal texture and desirable resin lines, it had a strong fragrance resembling the smell of honey or concentrated sugar, and had a bitter and pungent taste after chewing.

All samples were analyzed by TLC (Fig. 1). 6,7-Dimethoxy-2-(2-phenylethyl) chromone, the blue spot at R_f: 0.37, was used as a standard constituent by TLC (ST, shown in Fig. 1). Both the chemically induced agarwood and wild agarwood displayed bright blue spots in the same position, while the characteristic spots were weak or absent in the TLC analysis of A1, A2, and CK. After staining with 5% vanillin–sulfuric acid and heating to 105 °C, the spot at R_f: 0.25 was identified as a characteristic spot for agarwood. Based on empirical evidence, characteristic spots with deeper color correlate with higher quality agarwood. These results showed that the quality of the induced agarwood was similar to that of wild agarwood, and better than that of the agarwood induced by inoculating *M. flavolivens* or trunk breaking.

Generally, high-quality agarwood has high resin content and high ethanol-soluble extract content. According to Table 2, the average levels of ethanol-soluble extract (g/g) from the two chemically induced agarwood samples were 19.65% and 22.13%, respectively, far surpassing the requirement (10%) for traditional Chinese medicine listed in *Chinese Pharmacopoeia* [7]. These levels also exceeded that from a wild agarwood sample. The amounts of essential

Table 1
Origin of agarwood samples.

Code name	Origin
W1	Wild agarwood was purchased from a market in Hainan (Hainan, China)
W2	Wild agarwood was purchased from a market in Maoming (Guangdong, China)
C1	Induced agarwood in 20 months from *A. sinensis* trees *via* the whole-tree agarwood-inducing technology
C2	Induced agarwood in 20 months from *A. sinensis* trees *via* the whole-tree agarwood-inducing technology with another agarwood inducer
A1	Induced agarwood in *A. sinensis* trees after inoculating *Melanotus flavolivens* in 6 months
A2	Induced agarwood in *A. sinensis* trees after partial trunk breaking in 6 months
CK	Healthy wood of *A. sinensis* trees as control