Artificial and Wild Agarwood

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Agarwood is a highly economically important medicinal herb with widespread uses; however, the difference between the biological activities of artificial and wild agarwood is unclear.

Keywords: artificial agarwood ; wild agarwood ; antioxidant ability ; Anti-acetylcholinesterase activity ; Anti-α-glucosidase activity

1. Introduction

Agarwood is a medicinal herb produced by *Aquilaria* of the *Thymelaeaceae* family. The 21 *Aquilaria* species are mainly distributed in approximately 20 countries from India to Malaysia to Papua New Guinea [1]. In China, they are mainly distributed in Guangxi, Guangdong, Yunnan and Hainan, among which *Aquilaria sinensis* and *Aquilaria yunnanensis* are endemic [2]. According to records, the utilization history of agarwood can be traced back to more than 2000 years ago [3], and its unique components are commonly used in the fields of incense, medicine, and religion [4]. Under natural conditions, it is impossible for a healthy *Aquilaria* tree to contain agarwood. Only external stress factors, causing a tree to activate a defense response, and the accumulation of secondary metabolic substances lead to the formation of agarwood [5]. Thus, the generation of agarwood is coincidental, and agarwood formation may take decades of processing [6]. Furthermore, over the years, wild agarwood resources have been over-harvested [7], resulting in a scarcity of natural agarwood. To meet the market demand for agarwood, *Aquilaria* trees have been planted artificially on a large scale in China and Southeast Asian countries. At present, there are more than five hundred million *Aquilaria* trees [1], and for accelerating the agarwood production in *Aquilaria* plantations, physical, chemical, and biological methods have been developed to induce agarwood formation [8][9]. Natural agarwood has various biological activities such as antioxidant, hypoglycemic, anti-bacterial, and anti-inflammatory [10][11], making a resource with high potential for application as a natural active ingredient. Thus far, 443 components have been identified in agarwood, of which 197 compounds have been isolated [12]. Many of them have been assayed for their biological activities, such as (6S,7S,8S)-6,7,8-trihydroxyl-2-(3-hydroxyl-4-methoxylphenylethyl)-5,6,7,8-tetrahydro-4H-chromen-4-one, 6-hydroxy-2-(2-phenylethyl)chromone, (5S,7S,9S,10S)-(+)-9-hydroxy-selina-3,11-dien-12-al acetylcholinesterase inhibitory activity [13][14][15], and 6,7-dimethoxy-2-[2-(4-methoxyphenyl)ethyl]chromone having anti-inflammatory and α-glucosidase inhibitory abilities [16][17]. The Chinese Pharmacopeia has several requirements for the quality of agarwood [18], however, currently, it does not include biological activity. Moreover, many components of agarwood have not been identified and isolated, and not all isolated components have been measured for their biological activity capacity. There have been few studies on the overall activity capacity of agarwood, and there is little literature describing the difference between the bioactivity levels of artificial and wild agarwood. Therefore, it is difficult to evaluate the bioactivity of artificial agarwood, which is an important aspect to study.

2. Materials

Fungal inoculation experiments were conducted on healthy, more than 4 years old and ≥5 cm in diameter *A. sinensis* and *A. crassna* trees in Beihai and Pingxiang cities, Guangxi, China, at their breast height (**Table 1**).

Table 1. Sample formation.

Sample No.	Species Name	Inoculation Time	Number of Samples
C12	***A. crassna***	**12 months**	**N = 3**
C18	***A. crassna***	**18 months**	**N = 3**
A6	***A. sinensis***	**6 months**	**N = 3**
A12	***A. sinensis***	**12 months**	**N = 3**

Sample No.	Species Name	Inoculation Time	Number of Samples
A18	*A. sinensis*	18 months	N = 3

Whole plants were harvested after 6, 12, and 18 months; samples were collected and dried (**Figure 1**), and the obtained black resin was agarwood. The control group was black wild agarwood (YS) with a high oil content produced by *A. sinensis*, and it was purchased from the agarwood market in Hainan province, China.

Figure 1. Artificial and wild agarwood.

3. Antioxidation Ability

3.1. DPPH Free Radical Scavenging Capacity

The agarwood produced by both *A. sinensis* and *A. crassna* showed strong elimination of DPPH radicals. The clearance rate of DPPH free radicals tended to increase with increasing alcohol-soluble extract concentrations from 0.2 mg/mL to 2 mg/mL (**Figure 2**); thus, the scavenging rates showed some dose dependence. The IC_{50} values of the DPPH free radical by the artificial agarwood samples were 0.4127 mg/mL (C12), 0.5632 mg/mL (C18), 0.7487 mg/mL (A6), 0.5763 mg/mL (A12), 0.3902 mg/mL (A18), and of the wild agarwood, YS, was 0.3070 mg/mL. All batches (five) of the artificial agarwood had excellent DPPH free radical clearance rates. The IC_{50} value of DPPH radicals for A18 was the smallest among the sample groups, indicating that with 50% scavenging rate, its natural active ingredient was more specific and effective in clearing DPPH radicals than those of the others.

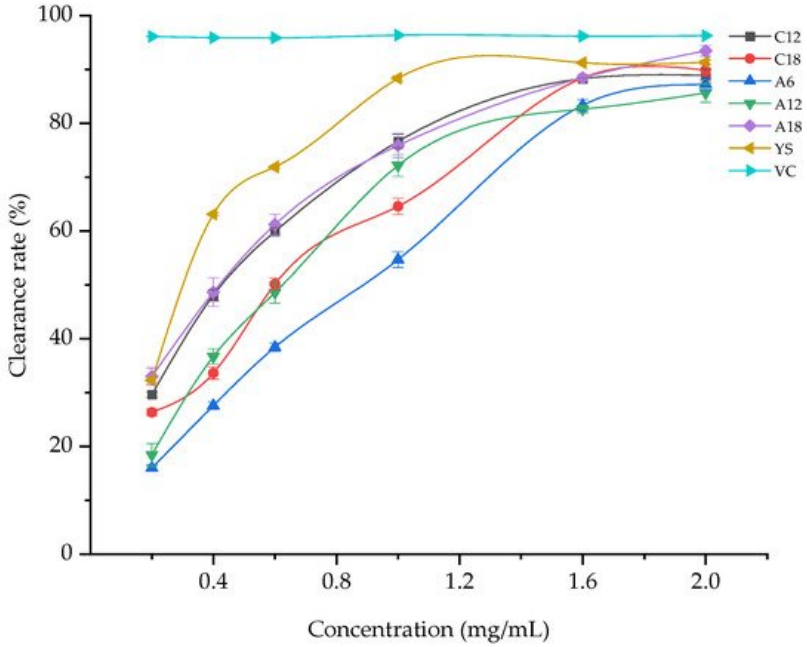

Figure 2. DPPH free radical clearance rate variation with extract concentration (C12 and C18 represented agarwood produced by *A. crassna* after 12 and 18 months of inoculation, respectively; A6–A18 represented agarwood produced by *A. sinensis* after 6, 12, and 18 months of inoculation, respectively; YS and VC represented wild agarwood and ascorbic acid, respectively).

The highest DPPH free radical scavenging rates were achieved when the alcohol-soluble extract concentration was 2 mg/mL. The corresponding rates of the samples and controls (VC and YS) followed the order VC (96.27 ± 0.09%) > A18 (93.45 ± 0.98%) > YS (91.34 ± 0.41%) > C18 (89.88 ± 1.07%) > C12 (88.92 ± 1.15%) > A6 (87.20 ± 1.03%) > A12 (85.62

± 1.76%) (**Table 2**), and all sample batches exhibited significant scavenging of DPPH free radicals. Moreover, the effect of VC on scavenging DPPH free radicals was significantly superior to all batches of agarwood ($p < 0.05$). The DPPH radical clearance rates of C12, C18, A6, A12, and A18 were 97.35%, 98.40%, 95.47%, 93.74%, and 102.31% of that of YS, respectively. The DPPH free radical scavenging effect of C18 was better than that of C12, whereas it was not significantly different from that of YS ($p < 0.05$). Moreover, the ability of A18 to scavenge DPPH free radicals was significantly higher than those of YS, C12, C18, A6, and A12 ($p < 0.05$). The results indicated that the fungal inducer not only improved the yield of this artificial agarwood and shortened the agarwood formation time but also made its ability to scavenge DPPH free radicals comparable to that of the wild agarwood. In terms of the inoculation time, the agarwood produced by 18 months of inoculation had a superior DPPH free radical scavenging rate to those produced by 6 and 12 months of inoculation. Interestingly, the DPPH free radical elimination effect of the *A. sinensis*-generated agarwood was better than that of the *A. crassna*-produced agarwood generated after 18 months of inoculation, and was even higher than that of the wild agarwood.

Table 2. DPPH free radical scavenging capacity of five batches and controls.

Samples	Alcohol-Soluble Extract Solution Concentration (mg/mL)	DPPH Free Radical Scavenging Capacity (%)
C12	2	88.92 ± 1.15 ^{d}
C18	2	89.88 ± 1.07 ^{c}
A6	2	87.20 ± 1.03 ^{d,e}
A12	2	85.62 ± 1.76 ^{e}
A18	2	93.45 ± 0.98 ^{b}
YS	2	91.34 ± 0.41 ^{c}
VC	2	96.27 ± 0.09 ^{a}

Each value represents the mean ± SD ($n = 3$); SD, standard deviation. At $p < 0.05$ according to one-way analysis of variation, mean values followed by different letters are significantly different from each other; mean values followed by one letter identical are not significantly different from each other.

3.2. ABTS^{+} Free Radical Scavenging Capacity

The clearance rate of ABTS^{+} free radicals increased with increasing mass concentration of the alcohol-soluble extract from 0.2 to 2 mg/mL (**Figure 3**). Moreover, the scavenging activity showed some dose dependence, with the scavenging rate initially increasing below sample concentration of 1.6 mg/mL and subsequently increasing gradually. The IC_{50} values of ATBS^{+} free radical by the artificial (five samples) and wild agarwood were 0.8401 mg/mL (C12), 0.9317 mg/mL (C18), 1.1710 mg/mL (A6), 0.8999 mg/mL (A12), 0.4472 mg/mL (A18), and 0.2368 mg/mL (YS), respectively. The IC_{50} value of ABTS^{+} free radicals for A18 was the smallest among the artificial agarwood samples, indicating that 50% scavenging rate contains a natural active ingredient that is more specific than those of the other samples.

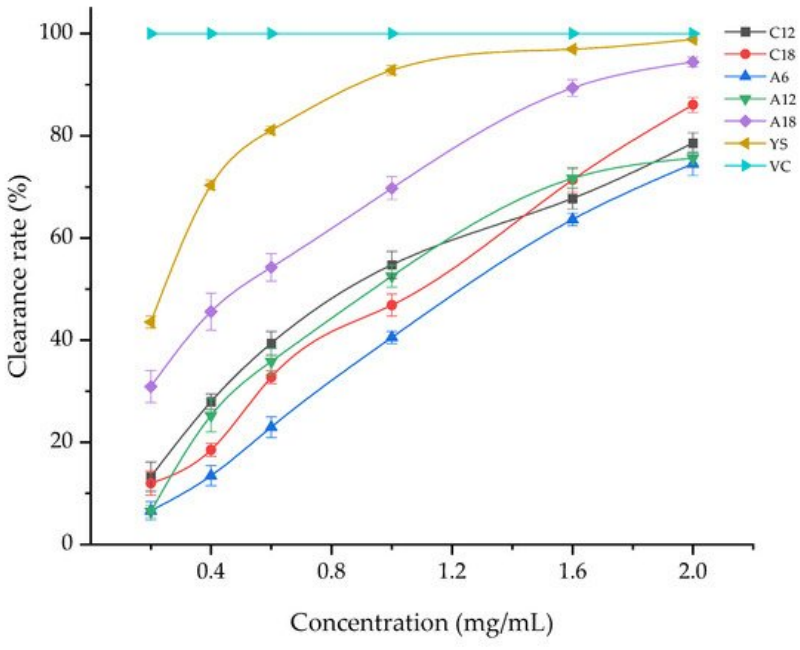

Figure 3. $ABTS^+$ free radical clearance rate variation with extract concentration (C12 and C18 represented agarwood produced by *A. crassna* after 12 and 18 months of inoculation, respectively; A6–A18 represented agarwood produced by *A. sinensis* after 6, 12, and 18 months of inoculation, respectively; YS and VC represented wild agarwood and ascorbic acid, respectively).

When the sample concentration was 2 mg/mL, the maximum $ABTS^+$ free radical scavenging rates of all sample batches (five) and controls followed the order VC (100%) > YS (98.87 ± 0.25%) > A18 (94.44 ± 0.94%) > C18 (86.06 ± 1.47%) > C12 (78.59 ± 2%) > A12 (75.62 ± 1.25%)> A6 (74.53 ± 2.31%) (**Table 3**). This trend suggests the presence of natural active ingredients with a better clearance effect on $ABTS^+$ free radicals. The $ABTS^+$ radical scavenging rates of C12, C18, A6, A12, and, A18 were 79.49%, 87.93%, 75.38%, 76.48%, and 95.52% of that of YS, respectively; the ability of C18 to clear $ABTS^+$ free radicals was significantly higher than that of C12 ($p < 0.05$). The $ABTS^+$ free radical clearance rate of A18 was significantly superior to those of C12, C18, A6, and A12 ($p < 0.05$). Interestingly, in terms of the inoculation time, $ABTS^+$ free radical clearance effects of the agarwood produced by both *A. sinensis* and *A. crassna* increased with the inoculation time. This suggests that the agarwood formed after 18 months of inoculation had a better ability to scavenge $ABTS^+$ free radicals than those formed after 6 and 12 months. This was particularly for the agarwood generated by *A. sinensis*, whose $ABTS^+$ free radical scavenging rate reached 95.52% of that of the wild agarwood, showing that the two rates are almost comparable.

Table 3. The $ABTS^+$ free radical scavenging capacity of five batches and controls.

Samples	Alcohol-Soluble Extract Solution Concentration (mg/mL)	$ABTS^+$ Free Radical Scavenging Capacity (%)
C12	**2**	**78.59 ± 2 ^{d}**
C18	**2**	**86.06 ± 1.47 ^{c}**
A6	**2**	**74.53 ± 2.31 ^{e}**
A12	**2**	**75.62 ± 1.25 ^{d,e}**
A18	**2**	**94.44 ± 0.94 ^{b}**
YS	**2**	**98.87 ± 0.25 ^{a}**
VC	**2**	**100 ^{a}**

Each value represents the mean ± SD ($n = 3$); SD, standard deviation. At $p < 0.05$ according to one-way analysis of variation, mean values followed by different letters are significantly different from each other; mean values followed by one letter identical are not significantly different from each other.

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