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Article

# Compositions and Antifungal Activities of Essential Oils from Agarwood of *Aquilaria sinensis* (Lour.) Gilg Induced by *Lasiodiplodia theobromae* (Pat.) Griffon. & Maubl

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A composição e atividade antimicrobiana dos óleos essenciais obtidos de madeira de ágar originária de *Aquilaria sinensis* (Lour.) Gilg induzido por agente biológico da madeira de ágar, *Lasiodiplodia theobromae* (F), foram caracterizadas e comparadas com madeira de ágar selvagem (W) e árvores saudáveis não inoculadas (H) como controles positivo e negativo, respectivamente. A composição química de F foi investigada usando cromatografia gasosa-espectrometria de massas (GC-MS). O óleo essencial de F mostrou uma composição similar de W, sendo rico em sesquiterpenos e constituintes aromáticos. No entanto, o óleo essencial de H era abundante em alcanos. Os óleos essenciais de F e W mostraram ser inibidores mais potentes de *L. theobromae*, *Fusarium oxysporum*, e *Candida albicans* do que o óleo essencial de H. Nossas descobertas demonstram pela primeira vez que o óleo essencial obtido da madeira de ágar originado de *A. sinensis* induzido por *L. theobromae* teve uma alta similaridade com o óleo essencial da madeira de ágar selvagem, tanto em composição química como em atividade antimicrobiana. Além disso, a estratégia de madeira de ágar induzida por fungos pode ser potencialmente aplicada em madeira de ágar e produção de óleo essencial em árvores do gênero *Aquilaria*.

The composition and antimicrobial activity of essential oils obtained from agarwood originating from *Aquilaria sinensis* (Lour.) Gilg induced by a biological agent of agarwood, *Lasiodiplodia theobromae* (F), were characterized and compared to those from wild agarwood (W) and uninoculated healthy trees (H) as positive and negative control, respectively. The chemical composition of F was investigated using gas chromatography-mass spectrometry (GC-MS). The essential oil of F showed a similar composition to that of W, being rich in sesquiterpenes and aromatic constituents. However, the essential oil of H was abundant in alkanes. Essential oils of F and W were more potent inhibitors of *L. theobromae*, *Fusarium oxysporum*, and *Candida albicans* than the essential oil of H. Our findings demonstrate for the first time that the essential oil obtained from the agarwood originating from *A. sinensis* induced by *L. theobromae* had a high similarity to that of the essential oil of wild agarwood, both in chemical composition and in antimicrobial activity. Furthermore, the strategy of agarwood induced by fungi could be potentially applied in agarwood and essential oil production in *Aquilaria* trees.

**Keywords:** agarwood, antifungal activity, *Aquilaria sinensis* (Lour.) Gilg, essential oil, GC-MS, *Lasiodiplodia theobromae* (Pat.) Griffon. & Maubl

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## Introduction

Agarwood is a resinous, fragrant wood, which is highly valued for its use in medicine, perfumes, and incense across Asia, Middle East and Europe.[1] Agarwood is produced by species of tropical trees of the genus *Aquilaria*, which are mainly distributed in South and Southeast Asia. Agarwood plays a role in Traditional Chinese Medicine for its sedative, carminative, and anti-emetic effects, and also as incense for religious ceremonies.[2] A large amount of agarwood is also consumed by distillation to obtain a fragrant oil, which is traditionally popular in the Middle East for blending with balm and perfume oil.[3,4] Agarwood is formed in wounded or microbe infected *Aquilaria* trees, but not in vigorously growing live trees.[5-10] Since 1938, many researchers have investigated agar formation and have reported the agar zones to be associated with mold and decay related fungi.[11-16] Among different fungal species reported to be associated with agar zones, most fungi seem to be of an aprophytic nature in different eco-geographical conditions.[17] However, little is known about the fungi associated with the development of disease symptoms and the resulting agarwood formation.

Our laboratory first isolated and identified the pathogen in *A. sinensis* dieback disease, *Lasiodiplodia theobromae* (Pat.) Griffon. & Maubl. Pathogenicity tests confirmed that *L. theobromae* was a natural pathogen of *A. sinensis* and induced the plant to produce agarwood.[18] In this study, in order to test the quality of the agarwood originating from *A. sinensis* induced by the fungal-inoculation method (F), its chemical composition and relative amount of essential oils were measured by gas chromatography-mass spectrometry (GC-MS), using wild agarwood (W) and healthy trees (H) as positive and negative controls, respectively. The antifungal activities of the essential oils derived from agarwood originating from *A. sinensis* were also determined.

## Experimental

### Plant materials

Four-year-old *A. sinensis* trees, which had been grown in a greenhouse in the Hainan Branch of the Institute of Medicinal Plant Development in Wanning, Xinglong County, Hainan Province of China, were used. *A. sinensis* trees were inoculated by making a vertical hole with a sterilized 0.4 cm drill to a depth of approximated 1 cm on the stem. A fungal disc of *L. theobromae* from a seven-day-old culture grown on potato dextrose agar (PDA) was placed over the wound, which was then covered with sterile, moist cotton and wrapped with Parafilm. Additional plants were treated similarly using only PDA and were used as the negative control. After 6 months, the fungal-inoculated (F) and control *A. sinensis* trees (H) were harvested for essential oil isolation. 20 cm long stems were collected and the hole was in the middle of each treated stem, and then the bark was stripped off and immersed in liquid nitrogen and stored at −80 °C for GC-MS analysis. For statistical analysis, data were calculated based on combined averages from five individual saplings (n = 6). Wild agarwood samples (W) were collected from Fengmu, Tunchang County, Hainan Province of China, and were identified by Prof Jian-He Wei. Three voucher specimens (201008526-8) are deposited at the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences.

### Essential oils separation

Three accurately weighed (100 g), dried and powdered samples (from F, W and H) were passed 20 mesh sieves, soaked in water overnight, and then submitted to hydrodistillation in a Clevenger apparatus at 100 °C for 12 h. The distillates were dried over anhydrous sodium sulfate and stored in a freezer at −20 °C until analysis.

### Gas chromatography-mass spectrometry analysis

The composition of the essential oil was determined using GC-MS analyses, which were performed using a Varian 450 gas chromatograph (Palo Alto, USA) equipped with a VF-5MS capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm) and a Varian 300 mass spectrometer with an ion-trap detector in EI mode at 70 eV in the m/e range 10-550 amu. The carrier gas was helium, at a flow rate of 1 mL $min^{-1}$. The injections were performed in splitless mode at 250 °C. 1 μL of essential oil solution in hexane (HPLC

**Table 1.** Materials used in this study

| Brief name[a] | Induction method | Characterization | Age |
|---|---|---|---|
| F | drill wound/fungal-inoculation | agarwood | 4 years |
| W | unknown natural factor | agarwood | > 20 years |
| H | drill wound | healthy trees | 4 years |

[a]F, W and H mean agarwood originating from *A. sinensis* induced by the fungal-inoculation method, wild agarwood and healthy trees, respectively.

grade) was injected. The operating parameters were the temperature program of 50 °C for 1 min, ramp of 10 °C $min^{-1}$ up to 155 °C (15 min), subsequent increase to 280 °C with an 8 °C $min^{-1}$ heating ramp, and keeping at 280 °C for 10 min. The components were identified by comparison of their mass spectra with the NIST 2002 library data for the GC-MS system, as well as by comparison of their retention indices (RI) with the relevant literature data.[19] The relative amount (RA) of each individual component of the essential oil was expressed as the percentage of the peak area relative to the total peak area. The RI value of each component was determined relative to the retention times (RT) of a series of $C_8$-$C_{40}$ *n*-alkanes with linear interpolation on the VF-5MS column.

### Antifungal activity

Two phytopathogenic fungi (*Lasiodiplodia theobromae* and *Fusarium oxysporum*) and one clinical fungus (*Candida albicans* ATCC10231) were used as test organisms in the screening. *L. theobromae* is a pathogen of *A. sinensis* dieback disease and was used as agarwood inducer.[18] *F. oxysporum* is a phytopathogenic fungus isolated from agarwood samples, which was identified based on a macroscopic analysis of the morphological properties of the mycelia and conidial spores, with the use of diagnostic keys.[20] *C. albicans*, a causal agent of opportunistic oral and genital infections in humans, was provided by the Institute for Food and Drug Control of China.

Antifungal activity of the essential oils was evaluated by the agar well diffusion method, according to described protocols with slight modifications.[21,22] *C. albicans* was grown in liquid potato dextrose (PD) medium overnight at 28 °C, and the diluted spore suspension ($10^5$ spores $mL^{-1}$) was prepared for assay. *F. oxysporum* and *L. theobromae* were maintained on PDA at 25 °C. The spores were prepared from 7-day-old cultures. A suspension of the tested fungi was prepared ($10^5$ spores $mL^{-1}$) and added (100 µL) into an agar plate, and dispensed uniformly onto the surface of the plate. Small wells were cut into the agar plate using a sterile cork-borer (6 mm), and 50 µL of the oil solution, at a concentration of 50 mg $mL^{-1}$ dissolved in dimethylsulfoxide (DMSO), was delivered into these wells. Negative controls were prepared using DMSO only. Fluconazole (200 µg $mL^{-1}$) was used as a standard since it is a clinically used anti-mycotic drug. Plates were incubated for 48 h at 35 °C for *C. albicans*, and at 25 °C for *L. theobromae* and *F. oxysporum*. The diameter of the inhibition zone around each well was then recorded in 4 different directions.

Minimum inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) values of essential oil against *L. theobromae*, *F. oxysporum*, and *C. albicans* were determined, based on a micro-well dilution method,[23,24] with some modifications. The spores of fungal strains were the same as those used for the agar well diffusion assay described above. The essential oils were dissolved in 10% DMSO, and were first diluted to the highest concentration (64 mg $mL^{-1}$) to be tested; then, serial two-fold dilutions were made in order to obtain a concentration range of 1-64 mg $mL^{-1}$ in 10 mL sterile test tubes containing PD broth. Next, 96 well plates were prepared by dispensing 100 µL suspension of the tested fungi ($10^5$ spores $mL^{-1}$) into each well. 100 µL of the stock solution of essential oil, prepared at a concentration of 50 mg $mL^{-1}$, were added into the first wells, and then 100 µl of their serial dilutions were transferred into six consecutive wells. The last well, containing 100 µL of PD broth without the compound and 100 µL of spore suspension from each strip, was used as negative control. The final volume in each well was 200 µL. Fluconazole (Institute for Food and Drug Control of China) was prepared in PD broth in the concentration range of 0.64-0.01 mg $mL^{-1}$ and was used as standard positive control. The plate was then covered with a sterile plate sealer. The contents of each well were mixed on a plate shaker at 300 rpm for 20 s and then incubated for 24 h at 35 °C for *C. albicans* and 25 °C for *L. theobromae* and *F. oxysporum*. Fungal growth in each medium was determined by reading the respective absorbance at 600 nm using a multimode microplate reader, Infinite M1000 (Tecan Trading AG, Männedorf, Switzerland) and was confirmed by plating 5 µL samples from clear wells onto PDA medium. The oil tested in this study was screened 3 times against each organism. MIC was defined as the lowest concentration of the respective compound capable of inhibiting the growth of fungi. MFC was defined as the lowest concentration of the essential oil that allowed no growth of fungi. Significant differences among means from triplicate analyses ($P < 0.05$) were determined by Duncan's multiple range test.

The antimicrobial activity of essential oils was analyzed by one-way ANOVA test using the statistical analysis system (SAS) Version 9 (SAS Institute, Cary, NC, USA). Significant differences among means from triplicate analyses ($P < 0.05$) were determined by Duncan's multiple range test.

## Results and Discussion

### Chemical composition of the essential oils

The yield of essential oils obtained after hydrodistillation of W, F and H was respectively 0.1158% (m/m), 0.0740% and 0.0079% (m/m). A total of seventy essential compounds

were identified from the three samples (Table 2, Figure S1 in Supplementary Information (SI) section). Forty two components were identified from W, representing 85.06% of the total volatiles, with the major constituents being sesquiterpenes and aromatic compounds, such as guai-1(10)-en-11-ol (6.35%), 2(3H)-naphthalenone, 4,4a,5,6,7,8-hexahydro-4a,5-dimethyl-3-(1-methylethylidene)-, (4ar-*cis*)- (6.36%), α-copaen-11-ol (10.84%), 1,3,5-trimethyl-2-(2,2,2-trifluoro-ethoxy)-benzene (13.40%) and baimuxinal (15.41%). Forty five components were identified in F, representing 89.64% of the total volatiles. The predominant compounds in the essential oil of F were sesquiterpenes and aromatic compounds, including α-copaen-11-ol (6.24%) and benzylacetone (19.51%). Fifteen components were identified from the essential oil derived from H, representing 95.41% of the total volatiles. Docosane (8.47%), hexacosane (10.08%),

**Table 2.** Chemical composition and relative amount of essential oil from W, F and H

| No.[a] | Compound | RI[b] | RI[c] | Relative amount / %[d] | | |
|---|---|---|---|---|---|---|
| | | | | W | F | H |
| | Aromatic compounds | | | 19.17 | 22.35 | 8.72 |
| 1 | Benzaldehyde | 982 | 977 | 0.36 | 1.62 | –[e] |
| 2 | *p*-Vinylanisole | 1160 | 1169 | 0.28 | 0.30 | – |
| 3 | Benzylacetone[f] | 1255 | 1262 | 3.60 | 19.51 | – |
| 4 | 1,3,5-Trimethyl-2-(2,2,2-trifluoro-ethoxy)-benzene | | 1766 | 13.40 | – | – |
| 5 | 2-[(4-Methoxyphenyl)methylene]-cyclohexanone | 1837 | 1847 | 0.32 | – | – |
| 6 | 1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester | 1877 | 1879 | 0.29 | – | – |
| 7 | Dibutyl phthalate | 1965 | 1966 | – | – | 2.08 |
| 8 | 4,4'-Methylenebis[2-(1,1-dimethylethyl)-6-methyl-phenol | | 2427 | 0.62 | 0.92 | 6.64 |
| 9 | 1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester | 2555 | 2548 | 0.30 | – | – |
| | Sesquiterpenes | | | 63.38 | 58 | 1.99 |
| 10 | (+)-Longifolene | 1418 | 1428 | – | 0.22 | – |
| 11 | Humulene | 1464 | 1468 | 1.44 | 1.28 | – |
| 12 | β-Chamigrene | 1485 | 1485 | – | 0.18 | – |
| 13 | α-Selinene | 1494 | 1498 | 4.08 | 3.49 | – |
| 14 | Isolongifolan-8-ol | 1520 | 1523 | 0.37 | 0.15 | – |
| 15 | 8,14-Cedranoxide | 1536 | 1539 | 0.78 | 0.50 | – |
| 16 | Spathulenol | 1557 | 1551 | 0.15 | – | – |
| 17 | Elemol | 1560 | 1560 | 0.13 | – | – |
| 18 | Alloaromadendrene oxide-(1) | | 1563 | – | 0.49 | – |
| 19 | 2,6-Dimethyl-10-methylene-12-oxatricyclo[7.3.1.0(1,6)]tridec-2-ene | 1576 | 1579 | 0.43 | 0.34 | – |
| 20 | 5β,7βH,10α-Eudesm-11-en-1α-ol | 1583 | 1588 | 0.59 | 1.34 | – |
| 21 | Caryophyllene oxide[f] | 1596 | 1595 | 1.08 | 3.39 | – |
| 22 | Santalol | 1617 | 1601 | – | 0.57 | – |
| 23 | Cedrenol | 1604 | 1610 | 2.43 | 1.80 | – |
| 24 | Isoaromadendrene epoxide | 1606 | 1612 | 0.32 | – | – |
| 25 | γ-Eudesmol | 1632 | 1636 | 1.74 | 1.45 | – |
| 26 | Octahydro-2,2,4,7α-tetramethyl-1,3α-ethano(1H)inden-4-ol | 1648 | 1640 | – | 0.44 | – |
| 27 | Hinesol | 1640 | 1648 | 1.19 | 1.35 | – |
| 28 | Agarospirol | 1646 | 1652 | 0.78 | – | – |
| 29 | (-)-Aristolene | 1654 | 1657 | – | 2.51 | – |
| 30 | α-Eudesmol | 1658 | 1660 | 0.27 | 1.35 | – |
| 31 | Cubenol | 1664 | 1666 | – | 3.30 | – |
| 32 | Guai-1(10)-en-11-ol | 1669 | 1669 | 6.35 | 1.04 | – |
| 33 | Eudesm-7(11)-en-4α-ol | | 1670 | 0.75 | 1.96 | – |
| 34 | Corymbolone | | 1676 | – | – | 1.99 |
| 35 | Aromadendrene oxide-(1) | 1674 | 1679 | 0.94 | 1.58 | |
| 36 | 6-Isopropenyl-4,8α-dimethyl-1,2,3,5,6,7,8,8α-octahydro-naphthalen-2-ol | 1678 | 1684 | 0.19 | – | – |
| 37 | α-Copaen-11-ol | 1686 | 1694 | 10.84 | 6.24 | – |
| 38 | Cycloisolongifolene | | 1699 | 1.15 | – | – |
| 39 | Aromadendrene oxide-(2) | 1705 | 1704 | – | 1.21 | – |
| 40 | 2,2,6-Trimethyl-1-(3-methyl-1,3-butadienyl)-5-methylene-7-oxabicyclo[4.1.0]heptane | | 1710 | – | 2.40 | – |

**Table 2.** continuation

| No.[a] | Compound | RI[b] | RI[c] | Relative amount / %[d] | | |
|---|---|---|---|---|---|---|
| | | | | W | F | H |
| 41 | *cis*-Z-α-Bisabolene epoxide | 1704 | 1713 | – | 4.68 | – |
| 42 | Baimuxinal | | 1716 | 15.41 | – | – |
| 43 | γ-Costol | 1732 | 1731 | – | 0.62 | – |
| 44 | 6-Ethenylhexahydro-6-methyl-3-methylene-7-(1-methylethenyl)-2(3H)-benzofuranone | | 1740 | – | 1.23 | – |
| 45 | Longifolenaldehyde | | 1741 | 0.64 | – | – |
| 46 | Longipinocarvone | 1747 | 1748 | 0.67 | – | – |
| 47 | Aristolone | 1762 | 1761 | – | 4.06 | – |
| 48 | (-)-Isolongifolol | 1781 | 1771 | 1.70 | – | – |
| 49 | 3,5,6,7,8,8α-Hexahydro-4,8α-dimethyl-6-(1-methylethenyl)-2(1H)naphthalenone | 1773 | 1775 | – | 0.96 | – |
| 50 | 3α,9β-Dihydroxy-3,5α,8-trimethyltricyclo[6.3.1.0(1,5)]dodecane | 1802 | 1803 | 0.60 | – | – |
| 51 | Nootkatone | 1820 | 1825 | – | 2.60 | – |
| 52 | (4ar-*cis*)-4,4α,5,6,7,8-Hexahydro-4α,5-dimethyl-3-(1-methylethylidene)-2(3H) naphthalenone | | 1828 | 6.36 | – | – |
| 53 | 3-Hydroxy-6-isopropenyl-4,8α-dimethyl-1,2,3,5,6,7,8,8α-octahydro-2-naphthalenyl acetate | | 1859 | – | 1.96 | – |
| 54 | 1,5-Dimethyl-3-hydroxy-8-(1-methylene-2-hydroxyethyl-1)-bicyclo[4.4.0]dec-5-ene | | 1969 | 1.43 | 2.23 | – |
| 55 | Eudesma-5,11(13)-dien-8,12-olide | | 1987 | 0.57 | 1.08 | – |
| | Alkanes | | | 0.54 | 7.00 | 83.08 |
| 56 | Eicosane | 2000 | 2002 | – | 0.51 | 1.65 |
| 57 | Heneicosane | 2100 | 2103 | 0.14 | 0.69 | 4.62 |
| 58 | Docosane | 2200 | 2204 | 0.18 | 1.17 | 8.47 |
| 59 | Tricosane | 2300 | 2304 | 0.22 | 1.42 | 11.44 |
| 60 | Tetracosane | 2400 | 2404 | – | 1.00 | 11.95 |
| 61 | Pentacosane | 2500 | 2504 | – | 1.02 | 10.34 |
| 62 | Hexacosane | 2600 | 2602 | – | 0.60 | 10.08 |
| 63 | Heptacosane | 2700 | 2705 | – | 0.59 | 10.68 |
| 64 | Octacosane | 2800 | 2803 | – | – | 6.88 |
| 65 | Nonacosane | 2900 | 2902 | – | – | 6.97 |
| | Others | | | 1.97 | 2.29 | 1.62 |
| 66 | Decana | 1208 | 1215 | 0.42 | – | – |
| 67 | Vinyl decanoate | | 1484 | 1.25 | – | – |
| 68 | (*S*)-3,4,4α,5,6,7,8,9-Octahydro-4α-methyl-2H-benzocyclohepten-2-one | 1593 | 1600 | 0.30 | – | – |
| 69 | *anti*,*anti*,*anti*-3,3,6,6,9,9,12,12-Octamethyl-pentacyclo[9.1.0.0(2,4).0(5,7).0(8,10)] dodecane | | 1684 | – | – | 1.62 |
| 70 | 3-[(3*E*,5*E*)-Deca-3,5-dienyl]cyclopentan-1-one | | 1769 | – | 2.29 | – |
| | TOTAL | | | 85.06 | 89.64 | 95.41 |

[a]Order of elution is given on VF-5MS; [b]RI indicates reported in literature for the VF-5MS column; [c]RI indicates the retention indices that were calculated against C8-C40 *n*-alkanes on VF-5MS column; [d]relative amount determined as the peak area relative to the total peak area; [e]not detected; [f]verified against a compound standard.

pentacosane (10.34%), heptacosane (10.68%), tricosane (11.44%), and tetracosane (11.95%) accounted for 62.97% of the total essential oil from H, which accounted for its smell and volatility.

Our investigation showed that the essential oil of W had similar components to that of F. Both these oils were rich in sesquiterpenes and aromatic compounds, which reached 82.55% in W and 80.35% in F. Twenty nine sesquiterpenes and eight aromatic compounds were identified in W, compared to thirty four sesquiterpenes and four aromatic compounds in F.

The essential oil of W and F had significantly different components from that of H. The essential oil of H contained no sesquiterpenes that had been identified in the oils of W and F. However, H was rich in alkanes, which accounted for 83.08% of the oil. Our previous report showed that after one week of storing, samples collected from healthy trees could produce up to 49% *n*-hexadecanoic acid as well as six sesquiterpenes, which accounted for 8.47%.

Agarwood causal agents could be divided into physical, chemical, and biological agents. Of these three agents, the biological method of agarwood induction, using fungi, is

recommended as it results in the progressive development of agarwood.[25] Studies have demonstrated that fungal species, such as *Aspergillus* sp., *Botryodiplodia* sp. (*Lasiodiplodia* sp.), *Diplodia* sp. *Fusarium bulbiferum*, *F. laterium*, *F. oxysporum*, *Penicillium* sp., *Pythium* sp., and *Trichoderma* sp., commonly infect *Aquilaria* species.[26] The effects of some isolates in agarwood formation have been tested by imitating the natural process. However, there are no reports concerning the quality of agarwood induced by the fungal-inoculation method. To our knowledge, this is the first report of fungal-inoculation induction of production of thirty-four sesquiterpenes and four aromatic compounds and agarwood formation in *A. sinensis*.

## Antimicrobial activities

We have suggested that plant defense mechanisms induced formation of agarwood.[27] Fungal infection activates the defense response mechanisms which in turn induce the formation of agarwood resulting in the biosynthesis of defense substances, such as sesquiterpenes in parenchyma cells. These phytoalexins accumulated and are secreted into the lumen of adjoining vessels via vessel-parenchymal pits, resulting in the formation of barriers, i.e., vessel occlusions. Both vessel occlusions and sesquiterpenes probably contribute to the physical restriction and chemical inhibition of microbes within vessels, consequently avoiding their spread. The number of vessel occlusions and the amount of sesquiterpenes increased with the period of infection time, ultimately leading to agarwood formation in the infected stem of *A. sinensis*.[27] Based on this hypothesis, the antifungal activity of the essential oil obtained from agarwood originating from F, W, and H was evaluated against *L. theobromae* (the biological agent of agarwood induction), *F. oxysporum* (a plant pathogen), and *C. albicans* (clinical fungi). The antifungal activity of the three essential oils from wild agarwood (W), induced by fungi (F), and uninoculated healthy trees (H) was evaluated by the agar diffusion method, as presented in Table 3. The essential oils of F and W (6.4 mg well$^{-1}$) were effective against all tested fungal strains. However, H demonstrated weak anti-fungal activity. The essential oil of W developed the largest zones of inhibition good activity against *C. albicans* and *F. oxysporum* with zones of inhibition comparable to those of fluconazole (200 μg well$^{-1}$). F was also active against *C. albicans*. Among the three fungi tested, *C. albicans* was found to be the most sensitive to all essential oils.

The antifungal activity of the three essential oils was assessed quantitatively by minimum inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) values, which are shown in Table 3. The MICs of W and F for all tested fungi ranged from 0.5 mg mL$^{-1}$ to 4 mg mL$^{-1}$, and MFCs ranged from 2 mg mL$^{-1}$ to 16 mg mL$^{-1}$, whereas H showed weak activity.

This is the first report concerning the antifungal activities of the three fungal strains of Chinese agarwood oil from *A. sinensis*. In previous studies, the essential oil from Chinese agarwood showed to have antibacterial activity against anti-methicillin–resistant *Staphylococcus aureus* (MRSA), *S. aureus*, and *Bacillus subtilis*, but not against *E. coli* at the maximum study concentration.[28,29] Novriyanti *et al.*[25] demonstrated that the ethyl acetate-soluble fraction of agarwood extract originating from *A. crassna* exhibited strong antifungal activity against *Fusarium solani* (a biological agent of agarwood induction). Wetwitayaklung *et al.*[30] found that the essential oil of agarwood (*A. crassna*) had antimicrobial activity against *C. albicans*. In this study, MIC values of the essential oils derived from F and W on *C. albicans* were 0.5 mg mL$^{-1}$

**Table 3.** Results of screening for antimicrobial activity of the 3 essential oils

| | Fungal strains | F | W | H | Fluconazole | DMSO | $H_2O$ |
|---|---|---|---|---|---|---|---|
| [a]AWD / (mm) | *C. albicans* | $7.94 \pm 0.11^{f}$ | $8.76 \pm 0.25^{e}$ | $0.53 \pm 0.09^{g}$ | $9.12 \pm 0.31^{d}$ | 0 | 0 |
| | *F. oxysporum* | $6.56 \pm 0.17^{f}$ | $7.12 \pm 0.17^{e}$ | $0.41 \pm 0.02^{g}$ | $7.58 \pm 0.19^{d}$ | 0 | 0 |
| | *L. theobromae* | $5.56 \pm 0.08^{e}$ | $5.74 \pm 0.35^{e}$ | $0.20 \pm 0.03^{f}$ | $6.73 \pm 0.15^{d}$ | 0 | 0 |
| [b]MIC / (mg mL$^{-1}$) | *C. albicans* | $1.0^{f}$ | $0.5^{e}$ | $16^{g}$ | $0.04^{d}$ | 0 | 0 |
| | *F. oxysporum* | $1^{e}$ | $2^{f}$ | $32^{g}$ | $0.08^{d}$ | 0 | 0 |
| | *L. theobromae* | $2^{e}$ | $4^{f}$ | $64^{g}$ | $0.16^{d}$ | 0 | 0 |
| [c]MFC / (mg mL$^{-1}$) | *C. albicans* | $2^{e}$ | $2^{e}$ | $32^{f}$ | $0.16^{d}$ | 0 | 0 |
| | *F. oxysporum* | $4^{e}$ | $4^{e}$ | $64^{f}$ | $0.32^{d}$ | 0 | 0 |
| | *L. theobromae* | $8^{e}$ | $16^{f}$ | $> 64^{g}$ | $0.32^{d}$ | 0 | 0 |

[a]AWD means agar well diffusion method. The diameters of the inhibition zone, including the well diameters, are 6 mm; [b]MIC and [c]MFC mean minimum inhibitory concentration and minimal fungicidal concentration, respectively. The values of the 3 oil samples are given in mg mL$^{-1}$. All results reported reflect the mean value of triplicate measurements. Means with different letters (d, e, f and g) are significantly different from each other at $P < 0.05$.

and 1 mg $mL^{-1}$, respectively. These values are reversed in Table 3, which is consistent with their report.

## Conclusions

Characterization of the essential oil obtained from the agarwood originating from *A. sinensis* induced by a fungal-inoculation method had a high similarity to that of the essential oil of wild agarwood, both in chemical composition and antimicrobial activity. This is the first reported analysis of essential oils from fungal-inoculation induced agarwood. Our data are consistent with the hypothesis that fungal infection activates plant defense mechanisms induced the biosynthesis of defensive substances, such as sesquiterpenes, which results in the formation of agarwood. It also indicates that the biological method of agarwood induction, using fungi, is one of the most promising inducers for agarwood industrial production.

## Supplementary Information

GC chromatograms of the three essential oils are available free of charge at http://jbcs.sbq.org.br as PDF file. Component numbers in the chromatogram correspond to those of Table 2.

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# Supplementary Information

## Compositions and Antifungal Activities of Essential Oils from Agarwood of *Aquilaria sinensis* (Lour.) Gilg Induced by *Lasiodiplodia theobromae* (Pat.) Griffon. & Maubl

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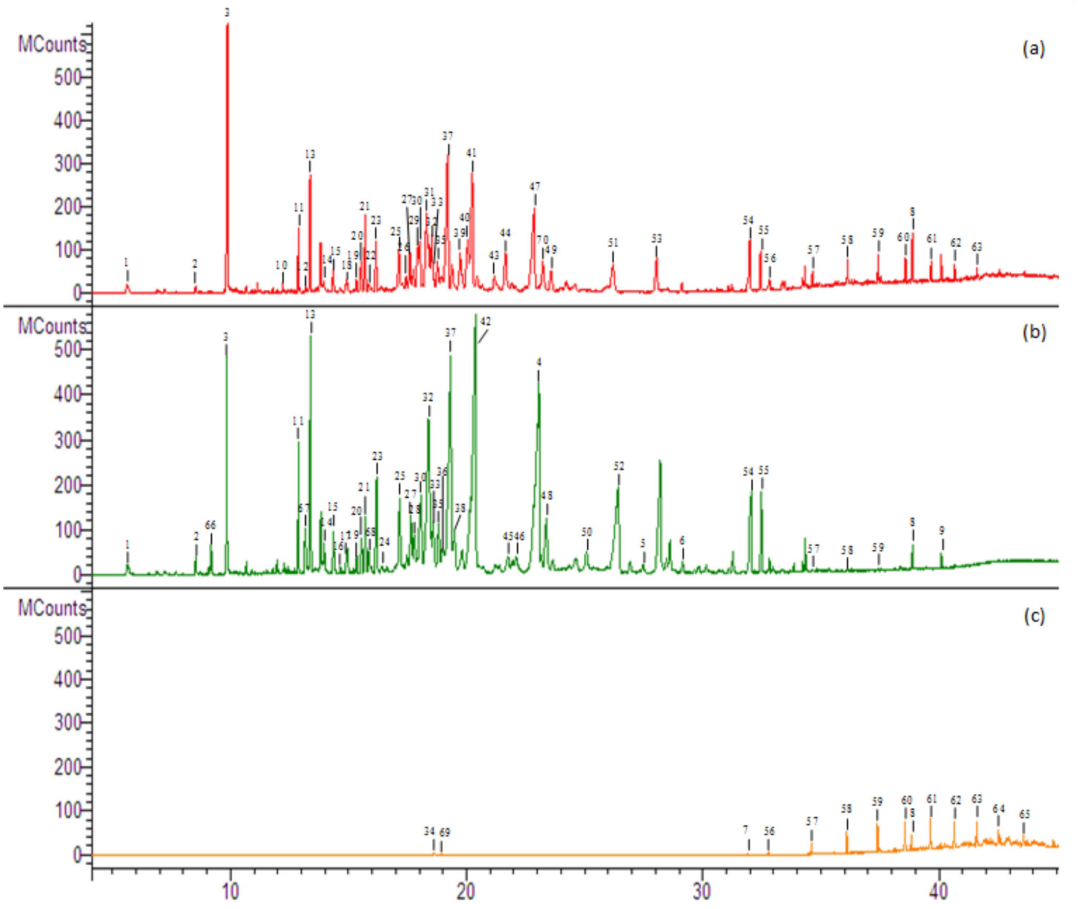


**Figure S1.** GC chromatograms of the three essential oils (a) W, (b) F and (c) H. Component numbers correspond to those of Table 2 and GC conditions are described in Experimental section of the article.

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