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ORIGINAL PAPER

Identification of chemical compounds in agarwood-producing species *Aquilaria malaccensis* and *Gyrinops versteegii*

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Abstract Agarwood is a non-timber forest product found in tropical rain forests. It is a black and fragrant resin valued for the perfume industry and demand continues to increase. However, the Indonesian agarwood-producing species, *Aquilaria malaccensis* and *Gyrinops versteegii* do not automatically produce such quality resin. Bio-induction technology or inoculation using *Fusarium solani* is usually applied to these species to trigger resin production. This research aims to identify agarwood compounds formed in seedlings and trees of *A. malaccensis* and *G. versteegii* after these species were inoculated with the fungus *F. solani*. The chemical compounds were identified by comparing the patterns of mass spectra fragmentation in the sample and in previous studies. Five groups of agarwood compounds were identified: (1) sesquiterpen group—*cis*-jasmone and aromadendrenepoxide; (2) chromones group—8-methoxy-2-(2-phenylethyl)chromen-4-one and newly-discovered chromone derivative, 7-(benzyloxy)-5-hydroxy-2-methylchromone found only in *G. versteegii*; (3) aromatic group—benzylacetone, guaiacol, *p*-ethylguaiacol, phenol, syringaldehyde, vanilin, furfuryl alcohol, and furfural; (4) fatty acid group—palmitic acid, oleic acid, and lauric acid; and, (5) triterpen group—squalene.

Keywords Agarwood constituent · *Aquilaria malaccensis* · *Fusarium solani* · *Gyrinops versteegii*

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Introduction

Agarwood resin is a non-timber forest product obtained from the agarwood-producing species *Aquilaria malaccensis* Lam. and *Gyrinops versteegii* (Gilg.) Domke. of Indonesia. It is a fragrant resin formed by wounding the tree and by fungal infection (Donovan and Puri 2004), a forest commodity that has a high demand in international perfume markets. *Aquilaria* and *Gyrinops* genera belong to the Thymelaeaceae, the most abundant plant family that produces agarwood (Gusmailina et al. 2010). *Aquilaria* is the most well-known genus and originates from Indomalesia, with *A. malaccensis* found abundantly in Sumatra and on the Kalimantan Islands (Lee and Mohamed 2016). *G. versteegii* originates from the Wallace Line (eastern part) of Indonesia (Subasinghe et al. 2012). These two genera have similarities in species distribution and taxonomy (Takeuchi and Golman 2002).

Agarwood resin is formed by fungi that infect the trees which respond by releasing a phytoalexin substance. This defense mechanism results in the accumulation of secondary metabolites in the area of the infection. The most

abundant chemical compound consists of a mixture of sesquiterpenes (52%), 2-(2-phenylethyl)-chromone derivatives (41%) (Lancester and Espinoza 2012), as well as other simple aromatic compounds (Naef 2011). However, not all agarwood-producing trees produce the scented black resin of natural agarwood. Therefore, bio-induction, a manufacturing technology to produce agarwood, has been introduced. Agarwood-producing trees were injected with biological agents such as fungi to imitate the mechanism of agarwood formation (Sitepu et al. 2011). Bio-induction involves endophytic fungi that extends the zone of agarwood formation to surrounding tissues and produces quality resin (Turjaman et al. 2016).

In order to determine the quality of agarwood formed, studies on the chemical composition of the resin have been carried out on *Aquilaria agallocha* Roxb. from India (Konishi et al. 2002), *A. malaccensis* from Indomalesia (Jong et al. 2014), *Aquilaria sinensis* (Lour.) Gilg. from China (Lin et al. 2010), *Aquilaria crassna* Pierre from China and Vietnam (Kumeta and Ito 2010), *Gyrinops walla* Gaertn. from India (Subasinghe et al. 2012) and *G. versteegii* from Indonesia (Tabata et al. 2003).

The objective of this research is to identify agarwood chemical compounds (thereby their qualities), formed in seedlings and mature trees of *A. malaccensis* and *G. versteegii* after inoculation with *Fusarium solani* (Mart.) Sacc.

Materials and methods

Sixty 5 month-old seedlings 30 cm high from the Cihideung nursery in Bogor, and six 5–7 year-old trees 15–20 cm diameter at the Arboretum Gaharu of *A. malaccensis* and *G. versteegii* were used in this study. The inoculant was obtained from FORDA and originated from Jambi, Sumatera Island and Malinau, Kalimantan Island. (Fig. 1)

Induction of agarwood

At the preparation phase, all equipment used were sterilized with 70% alcohol to prevent microbial contamination. The source of inoculant from each isolate was homogenized in a blender for 2–3 min.

Seedlings of *A. malaccensis* and *G. versteegii* were inoculated by making three transverse cuts on the bark 5 cm above the ground and inserting a cotton slice soaked with the inoculant.

For the trees, the inoculant was injected into 30 holes in each tree by making a transverse drill into the stem (about 1/3 of the stem diameter or 10 cm deep) and injecting the inoculant. The distance between the holes was 10 cm × 10 cm along the trunk, and each hole was given an inoculant dosage of 1 ml (Sitepu et al. 2011).

Sample preparation

The bark was removed from around the stem holes of seedlings and trees. The stems were sliced from the trunk of seedlings and trees at the incision locations or three holes that had been inoculated and had turned brown. Observations were conducted before inoculation, 2 months after inoculation and 6 months after inoculation. Samples harvested from several holes were mixed for each plant per inoculant and dried in an oven for 16 h.

Analysis of the chemical compounds

The chemical compounds produced after inoculation were identified using pyrolysis gas chromatography-mass spectrophotometry (py-GCMS) Shimadzu QP-2010 and Agilent 19091S-436. The resin was ionized using the gas chromatograph in an HP-5MS capillary column (silica 60 m × 0.25 mm × 0.25 µm). Oven temperatures ranged between 50 and 350 °C at an increasing rate of 15 °C/min for a constant flow mode. The condition of the front inlet with split method was: split ratio 50:1, temperature 280 °C,

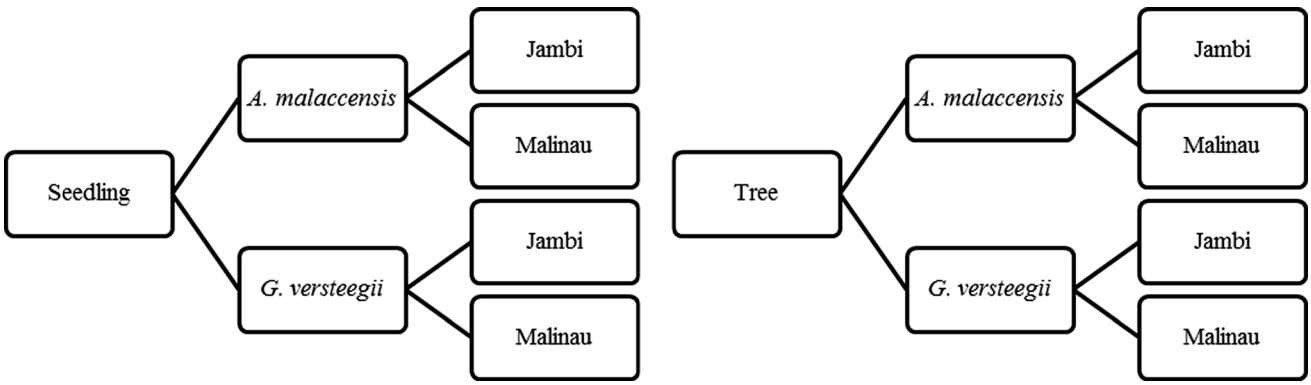

Fig. 1 Diagram of study design

helium gas rate 53.3 ml/min. This process was carried out for 50 min. The data included retention time, relative concentrations, and compound name. Data were analyzed and compared with the patterns of mass spectrophotometry from other studies (Mei et al. 2013) and WILEY 9 data library (Dharmadasa et al. 2013). Compound names were verified using the National Institute of Standards and Technology (NIST) database. The analysis was performed using MetaboAnalyst 3.0 online application.

Results and discussion

The analysis of the chemical compounds showed that there was a difference between healthy seedlings and trees, and those inoculated by the fungus. Healthy plant material had a group of compounds, including aromatic groups, fatty acids, other organic compounds, and glucose. In addition to these, a fourth group was found in the inoculated seedlings and trees and enriched with two main identifier groups of agarwood compounds i.e., sesquiterpenoid and derivative chromones. Agarwood constituent compounds, which were significantly present in inoculated seedlings and trees were guaiacol, oleic acid and palmitic acid (Fig. 2).

Guaiacol is an agarwood constituent compound which functions as a defense agent in *Aquilaria microcarpa* (Novriyanti 2010), and was also detected in the healthy plants. Guaiacol is a highly aromatic compounds often used as an ingredient in the perfume industry. It is also an intermediary compound for vanillin formation, and is an anti-bacterial and anti-parasitic agent (Li and Rosazza 2000). Palmitic acid was detected in artificial agarwood essential oil by making holes on the type *A. agallocha* (Bhuiyan et al. 2009). In previous reports, *A. malaccensis* was a synonyme for *A. agallocha* (Tamuli et al. 2005). *A. agallocha* was distributed in Bangladesh, eastern India, and Southeast Asia (Gibson 1977 in Bhuiyan et al. 2009). Oleic acid is one of the fatty acids which is a primary constituent in samples of natural and artificial agarwood from *A. malaccensis* (Faizal et al. 2017). Figure 2 presents the mass spectra of predominant compounds.

Chemical compounds seedlings

There were 104 compounds in *A. malaccensis* and *G. versteegii* species in which nine were identified as agarwood constituents. These were found in all samples and the principal compound was guaiacol with a concentration range of 0.6–8.0%, either in the controls or those inoculated from Jambi and Malinau. Two other dominant compounds detected in both species were palmitic acid and oleic acid with concentration ranges of 0.9–4.5% and 0.8–4.1%, respectively. The other compounds, which were found in both species, were *p*-ethylguaiacol, syringaldehyde and furfural. An agarwood identifier in *G. versteegii* with Jambi inoculant was 7-(benzyloxy)-5-hydroxy-2-methylchromone with a relative concentration of 1.6% (Table 1).

Chemical compounds in trees

There were 89 compounds found in the tree samples of which 16 were identified as agarwood constituents. The dominant constituents were palmitic acid and oleic acid, with concentrations of 0.6–19.6% and 0.5–17.5%, respectively. Five other compounds, which were also detected in both species, were benzylacetone, lauric acid, squalene, syringaldehyde, and furfural. However, the first three were detected only in inoculated trees.

Benzylacetone is one of the compounds that belongs to the constituents of agarwood in *A. microcarpa* (Novriyanti 2010). This compound, with a concentration range of

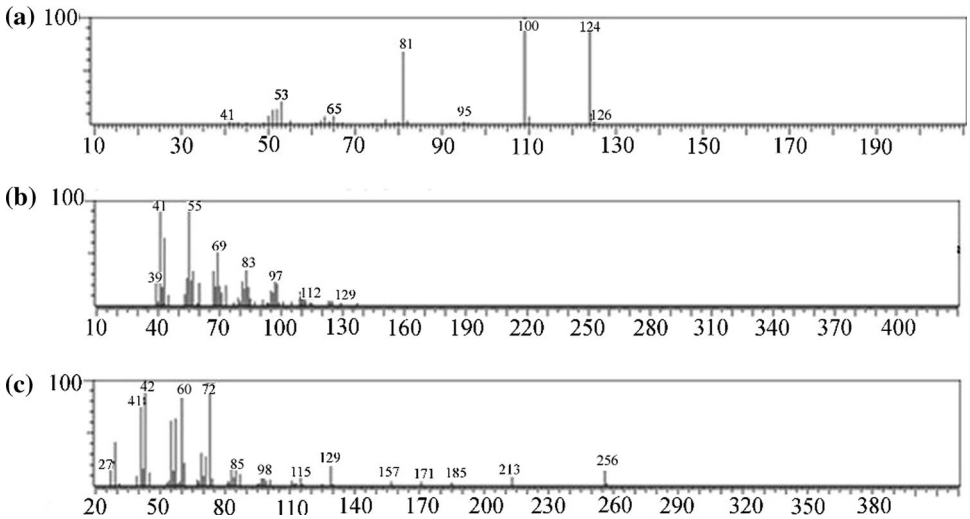

Fig. 2 Mass spectra of predominant compounds **a** guaiacol, **b** oleic acid, and **c** palmitic acid

Table 1 Relative concentrations of agarwood constituents identified in seedlings (%)

No.	Compounds	*A. malaccensis*					*G. versteegii*				
		Control	Jambi		Malinau		Control	Jambi		Malinau	
		0	2	6	2	6	0	2	6	2	6
	Chromones										
1	7-(Benzyloxy)-5-hydroxy-2-methylchromone	–	–	–	–	–	–	1.62	–	–	–
	Aromatics										
2	Guaiacol	0.62	8.02	3.76	2.43	3.46	0.63	2.71	5.40	6.23	3.79
3	*p*-ethylguaiacol	–	–	–	1.98	–	–	–	0.91	2.91	–
4	Phenol	–	–	–	1.08	–	–	–	–	–	–
5	Syringaldehyde	–	–	1.67	–	1.92	0.51	–	–	–	0.99
6	Vanillin	–	–	–	–	–	–	–	–	–	0.73
7	Furfural	0.96	–	–	–	–	0.95	–	–	–	–
	Fatty acids										
8	Oleic acid	–	1.51	0.87	4.09	1.42	–	0.85	–	2.47	–
9	Palmitic acid	–	4.50	0.91	3.21	1.10	–	–	1.57	–	0.90
	Total	1.24	14.03	7.21	12.79	7.90	2.09	5.18	7.88	11.61	6.41
	Non-agarwood	98.76	85.97	92.79	87.21	92.10	97.91	94.82	92.12	88.39	93.59
	Total	100	100	100	100	100	100	100	100	100	100

A. malaccensis and *G. versteegii*: agarwood producing tree; Jambi and Malinau: inoculant origin; 0, 2, and 6: inoculation periods in month

1.1–2.8%, was found in almost all the inoculated tree samples. Figure 3 shows the mass spectra of benzylacetone and was considered to be associated with the formation of agarwood resin (Chen et al. 2012). This compound was a major component of smog originating from Hong Kong's agarwood oil markets (Takemoto et al. 2008). A report by Jia and Yi (2018) also detected benzylacetone in agarwood using the thermal desorption-GCMS (TD-GCMS) method.

Lauric acid is an aromatic fatty acid and has been detected in *A. malaccensis* gaharu oil (Fazila and Halim 2012). This compound had a concentration of 0.9–3.4%, and was only identified in trees, not in seedlings, of both species. Another agarwood constituent found in other *Aquilaria* species was triterpen (Chen et al. 2012). The triterpen compound was squalene with a 0.3–0.6% concentration range, and accumulated in both tree species with the inoculant from Jambi. Squalene is a compound believed to be intermediate during sesquiterpen formed by *Fusarium* interaction with *A. malaccensis* (Sen et al. 2017).

Natural sesquiterpen is divided into thirty groups of compounds obtained from various plant sources. Two were found in this experiment from the groups patchoulolane and aromadendrene. Jasmone is a natural sesquiterpen included in the derivative patchoulolane (Fraga 2013), and *cis*-Jasmone was detected in *A. malaccensis* with the Jambi inoculant after 2 months. This compound has also been found in *Stellera chamaejasme* L. cell culture and named chamaejasmone (Qiao et al. 2012). In addition, aromadendrenepoxide was also detected in *A. malaccensis* with the Jambi inoculant after 2 months. Alloaromadendrene oxide is the stereoisomer of aromadendrenepoxide found in Malaysian agarwood (Jong et al. 2014). Another stereoisomer, isoaromadendreneepoxide was detected in agarwood oil from *A. malaccensis* originating from Malaysia (Tajuddin et al. 2013). Aromadendrene was detected at a concentration of 3.9% in the high quality agarwood samples in this study extracted using the direct thermal desorption (DTD) technique (Ismail et al. 2016). Figure 4 presents the mass spectra of the sesquiterpenoid derivatives group.

The chromone group derivative was 7-(benzyloxy)-5-hydroxy-2-methyl-4H-1-benzopyran-4-one with a 2.2% concentration in *A. malaccensis* trees with the inoculant from Jambi that had been induced for 2 months. The

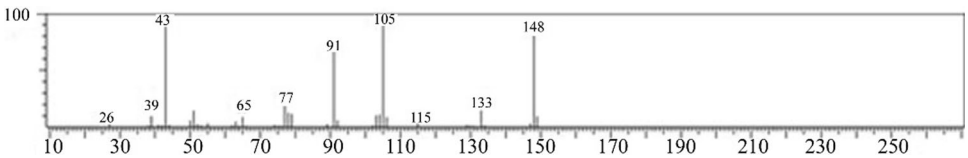

Fig. 3 Mass spectra of benzylacetone

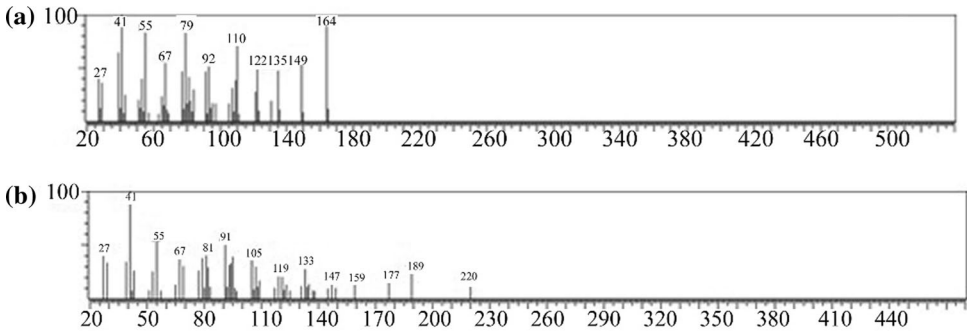

Fig. 4 Mass spectra of sesquiterpenoid derivatives **a** *cis*-Jasmone and **b** aromadendrenepoxide

chromone is a benzopyran formed by replacing the keto group on the pyran ring. The 4H-1-benzopyran-4-one chromone was also found as eight newly derived derivatives of chromones from *A. malaccensis* (Wu et al. 2012). The 8-methoxy-2-(2-phenylethyl)chromen-4-one with 0.3–2.3% concentration was detected only in *G. versteegii* induced by Jambi and Malinau inoculants, respectively. The same compound was also found in extracts of ether of *A. malaccensis* wood which had been infected for 6 months of inoculated and 1 year of inoculated by the fungus *Melanotus flavolivens* (Kumeta and Ito 2010) (Table 2). Figure 5 presents the mass spectra of the chromone derivatives group.

Agarwood constituent compounds

The *cis*-Jasmone, aromadendrenepoxide, and phenol compounds were detected only in *A. malaccensis*, whereas 8-methoxy-2-(2-phenylethyl)chromen-4-one and vanilin were only in *G. versteegii*. Both species had potential to produce high quality agarwood because they were able to accumulate agarwood identifier compounds. In addition, this was confirmed with the finding of new compounds that had not been detected in previous studies related to chromone derivatives in the form of compound 7-(benzyloxy)-5-hydroxy-2-methylchromone. The groups of the sesquiterpenes and chromones can be generated from agarwood exposed to heat so that it contains equivalent complex compounds to sesquiterpen, chromone pyrolysis products, pulp and lignin. The py-GCMS results show that the sesquiterpene compound group were not commonly detected in the study because most of these compounds had volatile properties (Rahayu et al. 2010).

Chromone is an isomer of coumarin which is a non-volatile compound of oxygen heterocyclic fractions (Baser and Demirci 2007). It is being investigated as a result of pyrolysis, whereas aromatic compounds are formed as a result of decomposition of the pulp and resin (Naef 2011). These compounds were more widely found in experimental samples because they were tolerant to thermal conditions. The sesquiterpenoid and chromone aromatic groups were also identified as constituents of agarwood (Naef 2011).

This group of compounds were detected in healthy and infected trees. Some aromatic group compounds, detected by the py-GCMS and confirmed as constituents of agarwood, consisted of benzylacetone, guaiacol, *p*-ethylguaiacol, phenol, syringaldehyde, and vanillin. These were also found in agarwood volatile compounds (Naef 2011). The *p*-ethylguaiacol was not found in healthy trees, only in the inoculated trees, although not all of them. This compound was reported as a simple volatile aromatic compound (Naef 2011). Similar to the *p*-ethylguaiacol, phenols were only found in inoculated samples of *A. malaccensis* seedling and tree samples. Phenols were detected at a concentration of 2.5% in the commercial agarwood samples extracted using a direct thermal desorption (DTD) process (Ismail et al. 2016). Syringaldehyde compounds were detected in seedlings and tree samples, and found in both healthy and inoculated samples. Vanilin, a flavour agent used in the perfume industry where aroma is required (Cowan 1999), along with guaiacol, a well-known constituent of agarwood, serve as the agents of defense in *A. microcarpa* (Novriyanti 2010). The groups of aromatic compounds appear as a result of the degradation of chromones and lignins, especially when gaharu wood is burned (Naef 2011). The aroma produced from burnt agarwood is different from healthy agarwood- producing trees. This is due to the deposit of aromatic compounds on gaharu resins accumulated in phloem tissues.

Other compound groups found in extracts from agarwood were fatty acids (Kallioinen et al. 2003). Fatty acids are commonly found in commercial types of agarwood from the genera *Aquilaria* or *Gyrinops*, e.g., *G. walla* (Subasinghe and Hettiarachchi 2013). A common fatty acid was formed as a result of the existence of the biotic attack-like fungi infection (Sen et al. 2017). The fatty acid compound group was also produced from the extraction process of essential oils by hydrofussion and was non-volatile (Handa et al. 2008). Non-volatile compounds such as fatty acids are one component of a compound that synthesizes volatile compounds (Goff and Klee 2006). Another

Table 2 Relative concentrations of agarwood constituents in tree samples (%)

No.	Compounds	*A. malaccensis*					*G. versteegii*				
		Control	Jambi		Malinau		Control	Jambi		Malinau	
		0	2	6	2	6	0	2	6	2	6
	Sesquiterpenes										
1	*cis*-jasmone	–	11.53	–	–	–	–	–	–	–	–
2	Aromadendrenepoxide	–	4.35	–	–	–	–	–	–	–	–
	Chromones										
3	7-(Benzyloxy)-5-hydroxy-2-methylchromone	–	2.19	–	–	–	–	–	–	–	–
4	8-Methoxy-2-(2-phenylethyl)chromen-4-one	–	–	–	–	–	–	–	0.34	2.28	–
	Aromatics										
5	Benzylacetone	–	1.06	–	2.27	2.23	–	–	–	2.77	1.73
6	Guaiacol	1.67	2.32	2.92	–	3.36	1.19	–	3.69	–	2.83
7	*p*-ethylguaiacol	–	–	–	–	–	–	–	1.97	–	–
8	Phenol	–	–	3.11	–	–	–	–	–	–	–
9	Syringaldehyde	0.6	–	–	–	–	–	–	1.28	–	2.14
10	Vanillin	–	–	–	–	–	–	–	0.76	–	–
11	Furfuryl alcohol	–	–	–	–	–	0.38	–	–	–	–
12	Furfural	1.52	–	–	–	–	0.84	–	–	–	–
	Fatty acids										
13	Lauric acid	–	1.39	–	2.44	–	–	3.43	–	0.86	–
14	Oleic acid	–	6.03	3.14	17.54	0.84	–	4.49	0.79	4.27	0.49
15	Palmitic acid	–	7.05	1.78	19.64	0.98	–	13.15	1.04	3.96	0.61
	Triterpen										
16	Squalene	–	–	0.62	–	–	–	–	0.34	–	–
	Total	3.79	35.92	11.57	41.89	7.41	2.41	21.07	10.21	14.14	7.80
	Non-agarwood	96.21	64.08	88.43	58.11	92.59	97.59	78.93	89.79	85.86	92.20
	Total	100	100	100	100	100	100	100	100	100	100

A. malaccensis and *G. versteegii*: agarwood producing tree; Jambi and Malinau: inoculant origin; 2 and 6: inoculation periods

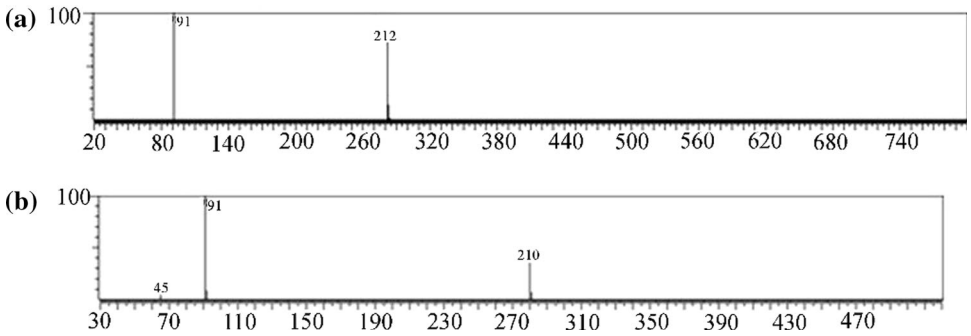

Fig. 5 Mass spectra of chromone derivatives: **a** 7-(benzyloxy)-5-hydroxy-2-methylchromen and **b** 8-methoxy-2-(2-phenylethyl)chromen-4-one

chemical constituent found from *Aquilaria* is triterpen (Santoso 2015). Triterpen were identified in this study, is a squalene with 1.0% concentration. It accumulated in *A. malaccensis* and *G. versteegii* trees with inoculant from Jambi at 6 months.

The concentrations of constituent compounds were analyzed by multivariate analysis using Principal Component Analysis scoring (PCA) and cluster analysis using the heatmap (Xia and Wishart 2016). PCA analysis simultaneously illustrates the relationship between variables. Constituent compounds that were presented in both types of growth rates for data distribution with 95% significance. It was demonstrated based on scatter data that still resided in one circle projection from the PCA analysis.

The elliptical shape showed the distribution of samples between variables. A larger elliptical shape showed that the distribution of samples had a wide distribution, and vice versa for a smaller oval. The relationship between variables can be observed by the distance between samples. The distance between the smaller sample showed that the samples had a strong relationship, and vice versa.

PCA of seedlings showed that the diversity is explained by 56.1% of PC 1 and 17.2% of PC 2. The total variability by these two components amounted to 73.3%. Agarwood samples from seedlings of *A. malaccensis* and *G. versteegii* with the Malinau inoculant after 2 months (SAM 2 and SGM 2) had a distribution in the opposite direction to the other samples but a close distance. This showed that the compounds of agarwood constituents contained in the SAM 2 and SGM 2 had a similar composition compared to other samples. This result indicates that the Malinau inoculant after 2 months had a similar effect on both species, and can be used as a distinguishing variable with other variables in seedlings (Fig. 6a).

The PCA of trees showed that diversity is explained by 64.7% of PC 1 and 17.9% of PC 2. The total variability explained by these two components amounted to 85.6%. The samples of *A. malaccensis* after two months with the Jambi inoculant (PAJ 2) had the greatest distance and in the opposite direction compared with the other samples. This indicates that the PAJ 2 sample can be used as a distinguishing variable from other samples. This is caused by the presence of the constituent composition of the group sesquiterpenoid and chromone derivatives with the greatest quantity than contained in the other samples (Fig. 6b). Based on the variance between PCA of seedlings and trees, the variance in the trees is larger than in the seedlings. This shows that there are some variables in PCA trees which, in this case, are agarwood constituents different from the other variables and have roles as agarwood identifier compounds.

The concentration of the compounds shown in Tables 1 and 2 are visualized by a heatmap which is a visualization of the distribution of the data described by color. The results showed that seedlings of the same species are in one cluster, although induced by different inoculants, Jambi and Malinau. The *A. malaccensis* seedling inoculant, Jambi (SAJ), is in a cluster with a seedling of *A. malaccensis* inoculant, Malinau (SAM). This is shown in furfural and palmitic acid compounds that are in one cluster. Both compounds had the same value changes between the two types of inoculants, but the change in the value of *A. malaccensis* seedlings, as indicated by the yellow colour, is higher than in G. *versteegii* seedlings indicated by the reddish orange. All of the constituents in the heatmaps of seedlings were divided into two clusters (Fig. 7a).

In trees, concentrations of compounds were divided into three clusters. *A. malaccensis* trees with Jambi inoculant (PAJ) was the most different cluster from the other two clusters. It was marked as a white color in the heatmap, to indicate that the compounds had the maximum change among the other samples. The samples detected with white was an agarwood identifier compound consisting of guaiacol, *cis*-jasmone, 7-(benzyloxy)-5-hydroxy-2-

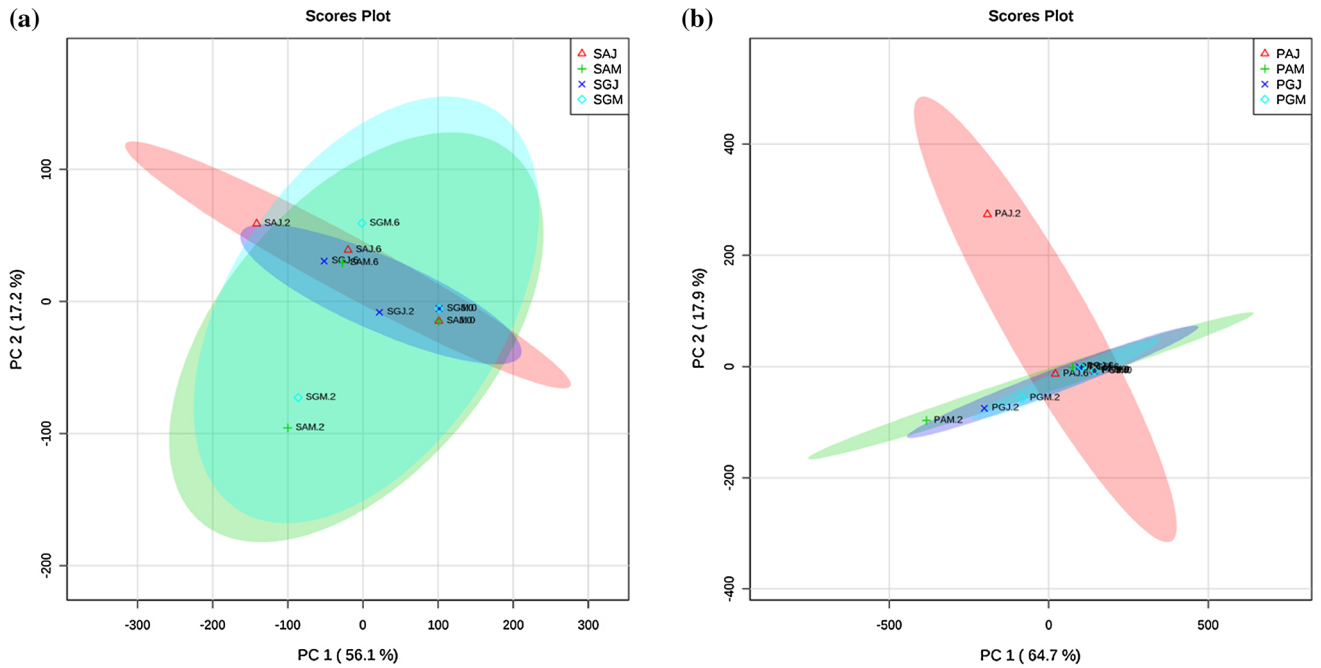

Fig. 6 The PCA of the agarwood constituent at *A. malaccensis* and *G. versteegii* **a** seedling and **b** tree

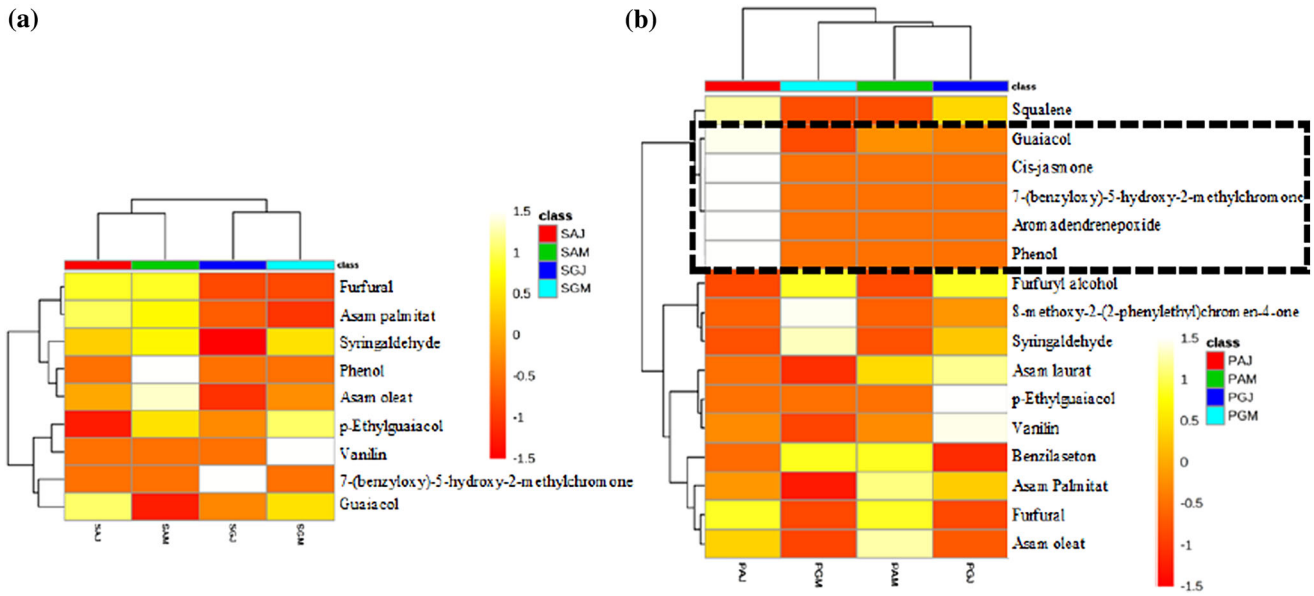

Fig. 7 Heatmap compounds of **a** seedlings and **b** trees

methylchromone, aromadendrenepoxide, and phenol (Fig. 7b). The heatmap indicates that not all inoculants of *F. solani* had the same effect on the different species of agarwood-producing trees. In addition, the different inoculant sources also produced differences in the quality of the compounds in agarwood.

Agarwood is a resinous compound produced by tree species due to biological and/or natural chemicals or by artificial infection. Agarwood is often used as an ingredient in various industries for the manufacture of perfumes or essential oils. Inoculated seedlings and trees have a group of compounds that are agarwood identifiers, namely, sesquiterpenoid and chromones. Sesquiterpenoid is one the secondary metabolite compounds of the terpene group that has 15 carbon atoms in the form of three 7 units of C5 fusion (Taiz and Zeiger 2002). These compounds are formed as a function of plant defense when there is an attack from an outside agent, such as a fungus, through the formation of fitoalexin compounds. The sesquiterpenoid were detected in this study was *cis*-Jasmone and aromadendrenepoxide.

A chemical component of agarwood is chromone. It is a compound found only in wood extracts of agarwood from tree samples. This compound plays a large role to provide a level of durability of scent when agarwood is burned or heated; it is the compound with the property of the organoleptic on the results of the combustion fumes (Naef 2011). The chromones, detected in this research, was 8-methoxy-2-(2-phenylethyl)chromen-4-one and 7-(benzyloxy)-5-hydroxy-2-methyl-4H-1-benzopyran-4-one.

The Forest Research and Development Center's research showed that *F. solani* is one of a significant endophytic fungi that form agarwood (Santoso 2014). *F. solani* isolate from Jambi was tested by the Forest Research and Development Center and found to have high growth level, specifically > 50 mm compared with other *Fusarium* isolates. The results of this research are in agreement with Santoso (2015) who reported that the source of the isolates significantly affected the formation of agarwood in *A. microcarpa* Baill. after 2 months inoculation, but there was no effect after 6 months. The two groups of compounds of the agarwood identifier were found more in the growth rate of the tree, especially at 2 months after inoculation. This was similar to fungal inoculation research on *A. microcarpa* after 2 months of inoculation and showed the highest agarwood formation symptoms such as color and fragrance. One of the reasons for this is believed to be related to the health of the tree that began to decrease after 2 months of inoculation (Rahayu et al. 2010). In addition, the results of the evaluation of *A. microcarpa* inoculated for 6 months indicate that the infection holes could be covered by wood tissue (Santoso 2015).

The limited agarwood compounds identified in seedlings could be due to leaf chlorosis symptoms that led to the death of plant tissues. This showed that seedlings were unable to resist fungal infections with high petogenitas level. For plants that produce agarwood, defoliation is an early symptom that the fungus has infected the plant (Rahayu et al. 2001). The next stage will be followed by a color change of the holes from white to brown to blackish, followed by the onset of aroma when heated.

Conclusions

Agarwood constituent compounds have been identified from seedlings and trees after inoculation, but more were deposited in trees. Agarwood identifier compounds that have been identified consisted of a sesquiterpenoid group such as *cis*-jasmone and aromadendrenepoxide. The other agarwood identifier compounds were chromone derivatives such as 8-methoxy-2-(2-phenylethyl)chromen-4-one and 7-(benzyloxy)-5-hydroxy-2-methylchromone. Other agarwood constituents, which were detected, were predominantly benzylacetone, guaiacol, palmitic acid and oleic acid. Agarwood constituent analysis needs to be done with the terraced fractionation method in order to get agarwood volatile compounds. The analysis was more specific and characterized using GCMS without pyrolisis to obtain more abundant volatile compounds in both species from Indonesia.

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