

# Integrated resin induction enhances Agarwood formation in *Gyrinops caudata*

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**Abstract.** Auri A, Dimara PA, Runtuboi YY. 2025. Integrated resin induction enhances Agarwood formation in *Gyrinops caudata*. *Biodiversitas* 26: 3758-3768. Natural agarwood formation occurs sporadically and unpredictably, making it difficult to achieve consistent quality and volume in commercial cultivation. This study aimed to investigate the synergistic effects of mechanical wounding, paraffin sealing, and biological induction using *Acremonium* sp. on agarwood formation in *Gyrinops caudata*. The investigation was conducted at an agarwood plantation in West Papua, Indonesia, using a split-plot design that incorporated various canopy pruning levels and four resin induction treatments. Additionally, quantitative and qualitative assessments of wood discoloration, aroma intensity, anatomical changes, and chemical composition were conducted over 6 months. The results of the integrated method, comprising mechanical drilling, biological inoculation with *Acremonium* sp., paraffin sealing, and 25% canopy pruning, resulted in the highest resinous wood area (694.2 mm<sup>2</sup>) and the greatest chemical diversity, with over 50 compounds identified via GC-MS. In contrast, untreated controls showed only 306.8 mm<sup>2</sup> of resin area and 15 compounds. "Integrated" in this context refers to the combination of physical wounding, microbial stimulation, and silvicultural stress designed to activate resin biosynthesis pathways synergistically.

**Keywords:** *Acremonium* sp., agarwood induction, GC-MS analysis, *Gyrinops caudata*, sesquiterpenes

## INTRODUCTION

Agarwood, renowned for its aromatic properties and significant economic value, is primarily derived from the *Gyrinops caudata* (Gilg) Domke and *Aquilaria* species within the Thymelaeaceae family. The formation of agarwood includes a complex biochemical response to biotic (pathogenic fungi) or abiotic (mechanical wounding) stress factors. This unique tree resin accumulates in response to stressors, presenting a combination of secondary metabolites, including sesquiterpenes and phenylpropanoids, which significantly influence the quality and fragrance of agarwood (Shivanand et al. 2022; Fauzi et al. 2024). The process of forming agarwood typically requires a considerable amount of time.

In Indonesia, the formation of natural agarwood occurs sporadically and unpredictably, making it challenging to achieve consistent quality and volume in commercial cultivation. This allows for the exploitation of natural forests to be carried out predictably, resulting in damage to the agarwood tree population. The process of forming is often random and influenced by various environmental factors, which complicate the establishment of a reliable agarwood supply. Mechanical injury and fungal infection are known to induce resin formation; however, the combined effects on the physiological and chemical characteristics of *G. caudata* remain less explored. Studies show that the interaction of environmental factors and stressors can lead to variations in resin quality, underscoring the need for a combined approach to grow and produce resin.

Various techniques have been developed to artificially induce agarwood formation, including chemical methods

using compounds such as sulfuric acid or sodium chloride, physical methods like drilling or stem wounding, and microbial inoculation using pathogenic or endophytic fungi (Zhou et al. 2021; Zhang et al. 2021; Falcon et al. 2025). Although chemical methods can be effective in the short term, residual chemicals may harm the environment and degrade agarwood quality. Mechanical methods often produce resin with low chemical richness. At the same time, microbial inoculation depends heavily on species compatibility and has shown inconsistent results in *G. caudata*, which responds differently compared to *Aquilaria* (Kadir et al. 2021). Therefore, an integrated induction approach combining mechanical, biological, and silvicultural stressors such as canopy pruning is considered more promising for synergistically activating secondary metabolic pathways. This method is not only more environmentally friendly but also has the potential to enhance both the quantity and quality of resin sustainably.

In addition to biological induction, silvicultural methods such as canopy pruning can modify microclimatic conditions, including light availability, which significantly influences the biosynthesis of secondary metabolites, including agarwood resin (Shivanand et al. 2022). In the context of agarwood, Auri et al. (2021a) determined that the crown pruning, together with *Acremonium* sp. inoculation, on agarwood formation. They concluded that although the direct visible effect of taper pruning on stem infectional spread could not be observed, it creates an overall favorable environment for microbial activity due to its influence on tree health and vitality (Auri et al. 2021b). However, substantial gaps remain in understanding the full spectrum of structural and functional changes in *G.*

*caudata* resulting from the combined induction method. A better understanding of this physiological response is crucial for designing effective and sustainable cultivation methods, as well as for enhancing the quantity and quality of agarwood (Liu et al. 2013; Fauzi et al. 2024).

The scientific literature has shown that stress-induced resin formation in agarwood-producing trees triggers a cascade of physiological responses, which vary between sites and differ according to environmental conditions, including light and microbial pressure. Sesquiterpenes and triterpenoids are consistently identified as the primary compounds that enhance fragrance and resin quality in agarwood (Herath and Jinendra 2023; Zhao et al. 2024). The high concentration of sesquiterpenes and triterpenoids, as well as their accumulation in wood tissue, influences the fragrant aroma produced by agarwood resin. Investigations combining structural, functional, and chemical perspectives in *G. caudata* are still limited. Only information is known about how canopy pruning may influence resin accumulation, particularly in interaction with induction methods. Therefore, this study aimed to evaluate the synergistic effects of mechanical wounding, paraffin sealing, and biological inoculation using *Acremonium* sp., combined with varying levels of canopy pruning, on resin production in *G. caudata*. The integrated application of these methods is hypothesized to significantly enhance resin yield and quality by modulating the physiological response and secondary metabolite production of the trees.

## MATERIALS AND METHODS

### Study area

This study was conducted at an agarwood plantation in Teluk Bintuni District, West Papua Province, Indonesia, from December 2018 to July 2019 (133°36'14.585" E - 2°6'2.026" S.). The experimental site was characterized by a tropical humid climate with an average daily temperature of 27°C, relative humidity between 75-85%, and no artificial environmental controls applied. The site was selected based on the ecological similarity to the natural habitat of *Gyrinops caudata*, including climate, soil type, and altitude. Fieldwork and laboratory analyses were conducted at the Silviculture and Forest Product Technology Laboratories, Faculty of Forestry, Universitas Papua, Indonesia.

### Procedures

#### *Plant material and experimental design*

A total of 60 healthy *G. caudata* trees, approximately 6 years old with a minimum Diameter at Breast Height (DBH) of 8 cm, were used in this study. Canopy pruning on all treatments was a split-plot design with pruning intensity as the main plot factor (no [0%], moderate [25%], and high [50%] canopies pruned to different light levels integrated into each block Plot (Auri et al. 2021b). This condition simulates light stress and redistributes assimilate allocation. The experiment employed a Completely Randomized Design (CRD) with the following treatments: P1 (Control): No treatment applied, P2 (Mechanical): Drilling of trunk using a 10 mm auger bit to a depth of 3

cm at 1 m above ground level, P3 (Mechanical): Drilling as in P2, followed by sealing, P4 (Integrated): Drilling and inoculation with fungi followed by sealing with paraffin wax. The biological agent used was *Acremonium* sp., isolated from naturally infected agarwood and cultured on Potato Dextrose Agar (PDA) medium. After 7 days of incubation at room temperature (28°C), the actively growing fungal mycelium was suspended in sterile distilled water and injected into the drilled holes (1 mL per site). A chemical sealing agent—paraffin wax (melting point 54-56°C)—was applied to the inoculated wounds to prevent desiccation and microbial contamination in the P4 treatment. No synthetic chemical elicitors were used in this study. Four treatments were tested on *G. caudata*, repeated five times at three canopy cover intensities, resulting in a total of 60 observation units. In this study, various levels of canopy pruning were anticipated to influence growth responses in the trees due to differing degrees of resource redistribution produced by each treatment. The growth of trees pruned for crown increment improved significantly in some studies, resulting in more diameter growth than the unpruned control (Auri et al. 2021a). This mechanical intervention induces a sense of wounding, where agarwood is formed as a defensive response, indicating that treating trees with drilling and correct sealing, followed by fungal inoculation, can actually aggravate resin production (Zhao et al. 2024).

#### *Mechanical and biological induction procedures*

During mechanical induction on the individual *G. caudata* tree, a 0.8-1.0 cm drill bit was used to bore one-third to half of the trunk perimeter depth into each tree, initiated at 10 cm above ground level (Zhang et al. 2022). Holes were prepared at three vertical zones (basal, middle, upper) with 10 per zone (30 holes per tree), spaced 5 cm apart. Biological inducer *Acremonium* sp. was grown on NN substrate (310 g/1000 mL) composed of rice powder, glucose, and potex solution, then incubated at 25°C for 10 days (Huang et al. 2023). The holes in the inoculated treatment group were filled with the resulting fungal suspension through injections, followed directly by sealing each hole with melted paraffin to maintain semi-anaerobic conditions, which are optimal for colonization and resin stimulation (Faizal et al. 2021). This method aimed to maximize agarwood formation through synergistic interaction between mechanical stress and biological induction, thereby enhancing both the quality and quantity of agarwood production (Overmans et al. 2024).

#### *Observation and data collection*

Three parameters, which were systematically measured to quantify the physiological responses elicited by the applied treatments, included the area of wood discoloration, aroma intensity, and anatomical changes. The discolored areas around individual holes were measured in square millimeters at 3 and 6 months post-treatment using digital calipers with image analysis software to obtain accurate measurements (Adimahavira et al. 2023). Three trained scorers assessed aroma intensity quarterly using an ordinal scale from 0 (no odor) to 3 (strongly smelling), and the

consistency of the olfactory analysis was checked by calculating the average scores per treatment group.

Wood samples (4 × 3 × 2 cm) through resin induction sites were used to examine anatomical changes. These samples were processed for slide preparation through fixation, sectioning on a sliding microtome, and staining with Lactophenol cotton blue to enhance the clarity of the structures under the microscope. At magnifications of 100× up to 400×, microscopic examination was performed, and measurements were carried out for different structures (vessel diameter, cell wall thickness). Additionally, the presence or absence of fungal hyphae was observed macroscopically as a trait to determine whether the study treatments could lead to alterations in wood structure (Auri et al. 2021a).

#### Chemical analysis (GC-MS)

Ground *G. caudata* wood (10 g) was extracted in acetone at room temperature for 24 hours, and the liquid extract was filtered. The solution obtained was analyzed using a Gas Chromatography-Mass Spectrometry (GC-MS) system from Agilent Technologies, following standard analytical protocols established for volatile compounds with minor modifications (Lopes et al. 2019). During identification, chemical compounds in the mass spectrum were evaluated against the NIST 05 and Wiley 275L databases (Santos et al. 2020). Moreover, quantitative data were presented as relative percentage concentrations of total compounds, using peak area normalization (Oktavianawati et al. 2024). The analysis explicitly centered on the identification of significant aromatic and aliphatic compounds related to agarwood generation, among which sesquiterpenes were classed into key categories along with triterpenoids as well as phenolic derivatives (Liao et al. 2018). The latter represents a valuable tool for providing insight into the aromatic and therapeutic properties of agarwood, with the aim of optimizing cultivation and induction practices.

#### Data analysis

A split-plot Analysis of Variance (ANOVA) model was employed for the statistical analysis of all quantitative data, including the area of discoloration, aroma score, and anatomical measurements. Tukey's Honestly Significant Difference (HSD) post-hoc test to compare differences among treatments. Statistical significance was determined at  $\alpha < 0.05$ . All analyses were performed using IBM SPSS Statistics version 26, for chemical profile data obtained from GC-MS. Principal Component Analysis (PCA) was conducted using R software (version 4.3.0) to visualize clustering patterns and assess multivariate variation among treatment groups. Additionally, a Pearson correlation analysis was conducted to investigate the relationship between changes in anatomical characteristics and the concentration of resin compounds. The results for the main binomial response were analyzed descriptively by comparing chemical profiles across treatments through grouping of GC-MS results according to compound class.

## RESULT AND DISCUSSION

### Infection area and wood discoloration

The results of environmental parameter measurements on cultivated land obtained type A rainfall, a temperature range of 20-30°C, air humidity of 77-85%, light intensity of 56-75%, and flat topography at an altitude of 100 m above sea level. The condition of the cultivated land is similar to that of the habitat of agarwood in natural forests.

*Gyrinops caudata* produces agarwood in response to biotic and mechanical stresses, where the level of wood discoloration serves as a key visual indicator for defending against pathogen infection. The experimental results showed that the inoculation of *Acremonium* sp. in each treatment group caused major changes in wood color. Regarding quantitative measurement, the average discolored area in treated trees was 694.2 mm<sup>2</sup> 6 months after pruning, compared to 306.8 mm<sup>2</sup> observed in the control group that received only drilling. Chromatic evaluations yielded  $\Delta E$  values ranging from 8.0 to 12.0, indicating visible darkening due to increased resin biosynthesis (Yan et al. 2019). The shifts in colorimetrically and spatially observed changes are consistent with the activation of biosynthetic pathways leading to the production of sesquiterpenes and 2-(2-phenylethyl)chromones, which represent the largest group defining high-grade agarwood quality (Sun et al. 2024). Effects of crown cover treatment, mechanical induction method, and *Acremonium* sp., on the average area of wood color change in *G. caudata* adult roots, and fungus inoculation are shown in Figure 1.

### Color chart of wood and analysis of infection rate

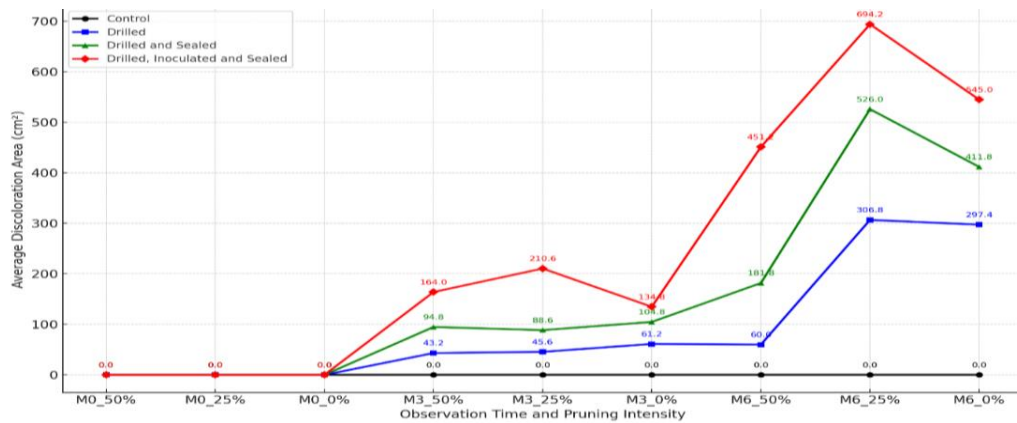
The infection area and resin were modulated by environmental factors, particularly canopy cover (significant interaction), in relation to the extent of infection (Figure 1). Trees with 25% and full canopy pruning exhibited the most sensitive wood color change, which could be attributed to optimal light permeation, leading to higher photosynthetic efficiency and secondary metabolite synthesis. Even with less needle removal (25% pruning), discoloration was more intensive and rapid in 3 months. Trees remaining unpruned (0% pruning) showed a higher accumulation of resin when examined 6 months after water stress, which proved temporal variability in physiological response security. This observation may be attributed to enhanced fungal activity during the early phase of agarwood formation under increased light exposure, where shaded conditions delay the plant defense response, leading to prolonged resin biosynthesis (Ali et al. 2023). The interactions demonstrate more broadly how microclimatic manipulation, specifically light regimes, can alter the time of year and the strength at which agarwood is produced. These signify the necessity of combining silvicultural methods to increase agarwood yield and quality in cultivated *G. caudata* stands (Vidurangi et al. 2022). The superior performance of the integrated treatment, which combined mechanical wounding, *Acremonium* sp. inoculation, and canopy pruning, can be attributed to its ability to trigger multiple layers of plant defense responses synergistically. Mechanical injury initiated local cell disruption, while fungal inoculation introduced biotic stress,

and pruning induced systemic physiological changes such as altered carbon allocation and hormonal signaling. Together, these stimuli likely activated Systemic Acquired Resistance (SAR) and elicited an oxidative burst that upregulated genes associated with secondary metabolite biosynthesis, including sesquiterpenes and chromones. This aligns with previous findings that dual or multiple stressors amplify defense-related gene expression and metabolite diversity compared to single treatments (Wang et al. 2016; Falcon et al. 2025). In particular, terpenoid biosynthesis is known to be enhanced by jasmonic acid and reactive oxygen species—both commonly elevated under combined biotic and abiotic stress conditions (Wang et al. 2021). The resulting chemical profile in the integrated treatment group thus reflects a more robust and diverse resin accumulation, consistent with higher-grade agarwood characteristics.

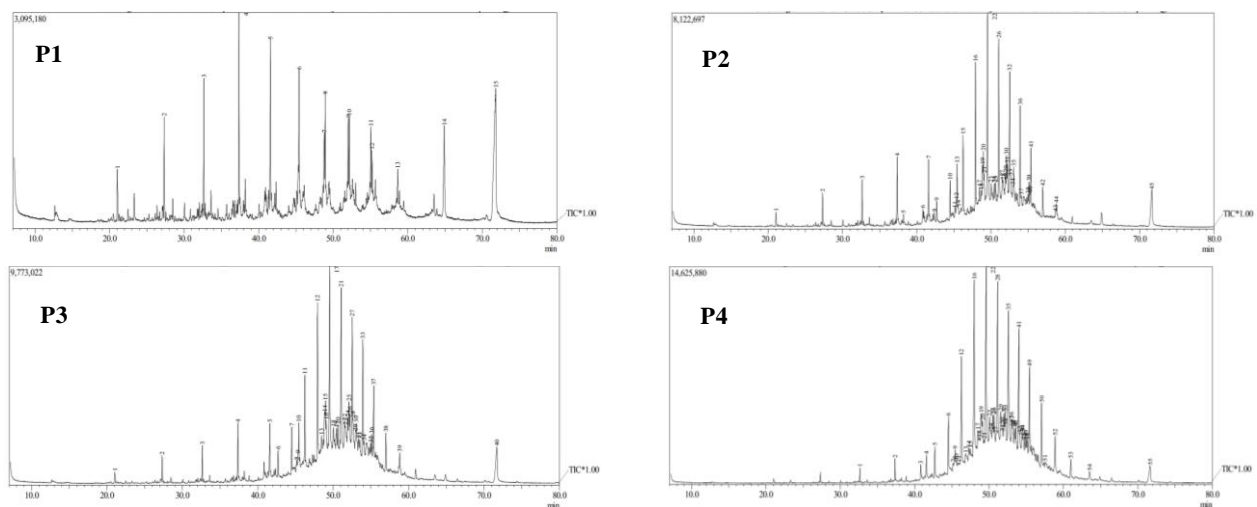
#### Chemical composition of Agarwood resin

GC-MS analysis revealed a significant enhancement in chemical diversity in the wood of *G. caudata* after fungal

inoculation, identifying 55 chemical compounds compared to only 15 in the control samples. Among these compounds, Benzaldehyde (10.3%), guaiacol (2.93%), coniferyl alcohol (2.70%), and eugenol (3.95%) were particularly prominent. The compounds are integral to the medicinal and aromatic qualities of agarwood, suggesting that fungal inoculation effectively stimulates biosynthetic pathways for sesquiterpenoids and phenolic compounds, which are critical for high-quality production (Huang et al. 2023; Zhang et al. 2024). The results demonstrate the potential of controlled fungal inoculation to enhance both yield and quality, particularly considering that only a small percentage of agarwood-producing trees (approximately 7–10%) naturally develop high-quality resin under stress conditions (Faizal et al. 2021; Zhao et al. 2024). GC-MS analysis of *G. caudata* control samples (P1) is shown in Table 1, and agarwood samples are presented in Figure 2.



**Figure 1.** Representative cross-section of *Gyrinops caudata* stem showing resinous wood formation after 3 months and 6 months of treatment. Treatments: P1: Control, P2: Mechanical treatment (drilling only), P3: Mechanical treatment (drilling + sealing), P4: Integrated treatment (drilling + *Acremonium* sp. + sealing with paraffin). Arrows indicate darkened resin zones



**Figure 2.** GC-MS agarwood samples to determine the chemical compounds formed. Treatments: P1: Control, P2: Mechanical treatment (drilling only), P3: Mechanical treatment (drilling + sealing), P4: Integrated treatment (drilling + *Acremonium* sp. + sealing with paraffin). Arrows indicate darkened resin zones

**Table 1.** GC-MS analysis of *Gyrinops caudata* control samples (P1)

Compound name	Molecular formula	Molecular weight (g/mol)	Retention time (minute)	Concentration (%)
Tetradecane	C14H30	198	21.03	3.43
Hexadecane	C16H34	226	27.31	4.81
Heptadecane	C17H36	240	32.64	5.88
Octadecane	C18H38	254	37.35	9.12
Nonadecane	C19H40	268	41.58	8.4
Heneicosane	C21H44	296	45.42	10.03
4-Decene, 2,2-dimethyl	C12H24	168	64.91	8.83
2,2-Dimethylpropane	C5H12	72	71.81	26.85

Source: GC-MS analysis (2022)

The application of controlled fungal inoculation, combined with effective canopy management, presents a viable solution to enhance agarwood production while alleviating the pressure on wild populations. Liu et al. (2024) reported that manipulating environmental conditions significantly affected resin yield, reinforcing the necessity of targeted inoculation strategies to maximize production efficiency. Furthermore, chemical profiles of artificially induced agarwood closely match those of wild agarwood. This signifies the pharmacological efficacy inherent in the synthetic resin, supporting the viability for medicinal and commercial applications (Huang et al. 2023; Zhang et al. 2024). Gas Chromatography-Mass Spectrometry (GC-MS) analysis of resinous wood from *G. caudata* treated with integrated induction methods revealed the presence of key sesquiterpenes and phenylethyl chromones associated with high-grade agarwood. Major compounds identified included  $\alpha$ -guaiene,  $\beta$ -agarofuran, squalene, hexatriacontane, and eicosane. Several unidentified phenylethyl chromones were also detected, as evidenced by retention times and fragmentation patterns, consistent with the core aroma components of agarwood. The presence of  $\alpha$ -guaiene and  $\beta$ -agarofuran has been previously reported in high-quality *Aquilaria malaccensis* and *Aquilaria crassna* resin (Naef 2011; Mailina et al. 2025), and is considered critical for the characteristic woody-balsamic scent valued in perfumery.

GC-MS analysis of control samples revealed the presence of various aromatic and hydrocarbon compounds, with 2,2-dimethylpropane (26.85%), Heneicosane (10.03%), and 4-decene, 2,2-dimethyl (8.83%) being the most abundant. These compounds indicate the activation of secondary metabolic pathways essential for terpenoid and aromatic compound biosynthesis, even in the absence of artificial induction (Adi et al. 2020; Subasinghe et al. 2022). The presence of heavier hydrocarbon compounds points to ongoing physiological adaptations during the early stages of resin accumulation, including vessel occlusion and cell wall thickening, signifying the response of the tree to microbial invasions or abiotic stress (Yu et al. 2020).

Microscopic examinations complement biochemical insights, revealing that while the structural integrity of untreated wood appears uniform, subtle anatomical changes occur, such as vessel dilation and cell activation. These changes may represent an initial phase of resin synthesis and storage, suggesting that agarwood formation is a gradual physiological process rather than an abrupt transformation (Adi et al. 2020; Faizal et al. 2021). Correlational data

between anatomical modifications and volatile compound profiles provide diagnostic tools for detecting early stages of agarwood formation, reinforcing the idea that successful agarwood induction depends on the correspondence of microbial activities with inherent defense mechanisms of the tree.

The integration of fungal inoculation and canopy management enhances agarwood production and promotes sustainability by alleviating pressure on natural resources. This shows the importance of understanding the biochemical and anatomical response in *G. caudata* to optimize agarwood yield in an effective and environmentally responsible manner.

GC-MS analysis of *G. caudata* wood subjected to mechanical drilling showed an array of chemical compounds representing an active metabolic response to mechanical injury. A total of 18 compounds were identified, reflecting the dynamic profile of secondary metabolites characteristic of the early stages of agarwood biosynthesis. GC-MS analysis of *G. caudata* drilled samples (P2) is shown in Table 2; the most abundant compound detected was Dotriacontane (C32H66). This is associated with the initiation of secondary metabolite synthesis and acts as a biochemical signal in response to stress, signifying the activation of pathways crucial for the biosynthesis of sesquiterpenes, which are primary components of agarwood fragrance (Wang et al. 2018; Yu et al. 2021).

Complementing the presence of 2-Nonanone, several hydrocarbons were detected, including Tetradecane, Hexadecane, and Heptadecane, with concentrations related to previous investigations suggesting that the hydrocarbons might serve as metabolic intermediates during resin formation (Subasinghe et al. 2021; Lukman et al. 2023). The identification of higher molecular weight compounds such as Hexacosane (C26H54), Octacosane (C28H58), Heptacosane (C27H56), and Heneicosane (C21H44), with concentrations ranging from 0.53% to 1.77%, signifies progressive biochemical accumulation. These heavier alkanes tend to contribute to structural defense mechanisms, reinforcing cell walls and potentially functioning as carriers for other resin constituents.

Longer-chain alkanes, including Dotriacontane (C32H66) and Hexatriacontane (C36H74), have been observed at concentrations of 10.84% and 1.16%, respectively, and serve as markers of advanced resin deposition, correlating with anatomical adaptations such as cell wall thickening (Lukman et al. 2023). The presence of Squalene (C30H50)

at 0.52% reinforces the inclusion of triterpenoid pathways in response to mechanical injury, while Phytane (C<sub>20</sub>H<sub>42</sub>) and various alkanes suggest a diversification toward lipid-based secondary metabolites, enhancing the properties of the resin (Wang et al. 2018; Yu et al. 2021).

Other compounds, such as 2-Methyltridecane and Pentadecane, with concentrations of 1.52% and 1.03%, may correspond to theories suggesting a connection to signaling or defense priming processes. These compounds contribute to the fragrance and characteristics of the developing agarwood (Manurung et al. 2021). Generally, the distribution of compounds shows a transition from volatile early-response molecules to heavier, more stable hydrocarbons associated with continual resin maturation.

Based on the results, mechanical drilling induces a robust biochemical response, leading to a foundational metabolic shift that pairs effectively with biological factors, such as fungal inoculation, to optimize agarwood formation. GC-MS profile of *G. caudata* subjected to mechanical stress encapsulates a multi-phase biochemical defense strategy, ranging from the release of small volatile alkanes to the accumulation of terpenoid intermediates and heavier hydrocarbons, signifying the essential role of mechanical stress in initiating agarwood biosynthesis (Wang et al. 2018; Peng et al. 2020).

GC-MS analysis of *G. caudata* wood treated through mechanical drilling and sealing presented a complex profile of aromatic hydrocarbons and volatile compounds, providing insights into the biochemical response of this tree species to combined physical injuries and enclosed microenvironmental conditions. A total of 18 compounds were detected, ranging from lightweight aliphatic volatiles to high-molecular-weight alkanes and triterpenoid precursors. GC-MS analysis of *G. caudata* drilled and Sealed samples (P3) is shown in Table 3. The result that Eicosane (C<sub>20</sub>H<sub>42</sub>)

was the most abundant compound, at 11.75%, showed an association with resin accumulation and metabolic stabilization (Shivanand et al. 2022).

Following eicosane, 2,2-dimethylpropane (C<sub>5</sub>H<sub>12</sub>) was present at 3.85%, a low-molecular-weight compound known for its role in early volatile signaling, which suggests a rapid defense response to physical disturbance (Shivanand et al. 2022). These two compounds represent different phases of resin development, where eicosane is reflective of matured resin, and 2,2-dimethylpropane relates to biochemical activation processes. Additionally, significant amounts of Hexatriacontane (3.57%), Tetracosane (2.74%), and Hexadecane (2.33%) contribute to the chemical defense and fragrance profile of developing agarwood, acting as essential components in lipid-based protective layers of the resin (Naef 2011).

The treatment group has a significant distribution of aliphatic hydrocarbons, including Dodecane and branched compounds such as 2-Methylnonadecane, with concentrations ranging from 0.60% to 1.10%. These branched and cyclic hydrocarbons reflect oxidative and enzymatic processes triggered by mechanical sealing, indicating chemical diversification in the resin synthesis pathways (Begum et al. 2024). The confined microenvironment generated by sealing appears to promote internal metabolic cycling and the retention of volatile intermediates, enhancing resin production.

Mid-to-heavy chain hydrocarbons were identified, including Heptadecane (1.35%), Heptacosane (1.04%), Nonadecane (2.52%), and Octadecane (0.77%), which suggests the gradual condensation and polymerization of resin precursors. These compounds play a structural role in the resin matrix and contribute to the chemical robustness of agarwood, reflecting a persistent response to physical stress (Shivanand et al. 2022).

**Table 2.** GC-MS analysis of *Gyrinops caudata* drilled samples (P2)

Compound name	Molecular formula	Molecular weight (g/mol)	Retention time (minute)	Concentration (%)
Tetradecane	C <sub>14</sub> H <sub>30</sub>	198	21.036	0.92
Hexadecane	C <sub>16</sub> H <sub>34</sub>	226	27.317	1.55
Heptadecane	C <sub>17</sub> H <sub>36</sub>	240	32.645	1.83
Octadecane	C <sub>18</sub> H <sub>38</sub>	254	37.363	3.04
Eicosane	C <sub>20</sub> H <sub>42</sub>	282	38.207	0.4
Nonadecane	C <sub>19</sub> H <sub>40</sub>	268	41.588	3
Hexacosane	C <sub>26</sub> H <sub>54</sub>	366	44.495	1.77
Dotriacontane	C <sub>32</sub> H <sub>66</sub>	450	49.515	10.84
Pentadecane	C <sub>15</sub> H <sub>32</sub>	212	49.987	1.03
Hexatriacontane	C <sub>36</sub> H <sub>74</sub>	506	50.427	1.16
2-Methyltridecane	C <sub>14</sub> H <sub>30</sub>	198	51.942	1.52
Heneicosane	C <sub>21</sub> H <sub>44</sub>	296	52.197	1.56
Squalene	C <sub>30</sub> H <sub>50</sub>	410	53.055	2.2
Cyclohexyl-eicosane	C <sub>26</sub> H <sub>52</sub>	364	54.075	0.58
Octacosane	C <sub>28</sub> H <sub>58</sub>	394	54.966	0.53
Heptacosane	C <sub>27</sub> H <sub>56</sub>	380	55.086	1.08
Phytane	C <sub>20</sub> H <sub>42</sub>	282	58.658	0.42
2,2-Dimethylpropane	C <sub>5</sub> H <sub>12</sub>	72	71.636	5.29

**Table 3.** GC-MS analysis of *Gyrinops caudata* drilled and sealed samples (P3)

Compound name	Molecular formula	Molecular weight (g/mol)	Retention time (minute)	Concentration (%)
2,2-Dimethylpropane	C5H12	72	21.047	3.85
2-Methylnonadecane	C20H42	282	32.649	1.1
Dodecane, 2-cyclohexyl-	C18H36	252	41.591	1.03
Dodecane, 3-methyl-	C13H28	184	44.509	0.6
Dotriacontane	C32H66	450	45.142	1.08
Eicosane	C20H42	282	45.325	11.75
Heneicosane	C21H44	296	45.442	1.4
Heptacosane	C27H56	380	48.867	1.04
Heptadecane	C17H36	240	49.538	1.35
Hexadecane	C16H34	226	50.451	2.33
Hexatriacontane	C36H74	506	51.633	3.57
Nonadecane	C19H40	268	52.218	2.52
Octadecane	C18H38	254	52.625	0.77
Pentadecane	C15H32	212	53.069	1.45
Squalene	C30H50	410	53.549	2.05
Tetracosane	C24H50	338	55.112	2.74
Tetradecane	C14H30	198	55.387	0.68
Tridecane	C13H28	184	71.723	2.17

Several high-molecular-weight compounds were identified, including Squalene (C30H50) at 2.05%, a key precursor in terpenoid biosynthesis associated with mechanical stress and semi-anaerobic conditions induced by sealing (Herath and Jinendra 2023). Pentadecane (1.45%) and Tridecane (2.17%) contribute as intermediates between light volatiles and fully polymerized resin compounds, representing an enhanced progression of hydrocarbon development due to mechanical sealing.

When comparing the sealed treatment with the open-drilled condition, the sealed treatment leads to a higher concentration and diversity of mid-to-heavy hydrocarbons. This suggests an optimal environment for resin maturation and volatile retention, reducing oxidative degradation and enabling persistent biosynthesis, as evidenced by the retention of Eicosane and Hexatriacontane (Naziz et al. 2019). The anatomical response supporting enhanced vessel occlusion and parenchymal thickening correlates with the observed chemical profile adjustments (Herath and Jinendra 2023).

GC-MS results from the mechanically drilled and sealed wood show a dynamic array of aromatic hydrocarbons. The interplay of volatile compounds, mid-weight alkanes, and resin-associated substances reflects a transitional stage of agarwood formation driven by physical injury and confined environments. Mechanical stress can only partially simulate the environment necessary for resin biosynthesis, while the full biochemical complexity may require additional biotic stimuli, such as fungal inoculation, to complete the metabolic cascade of agarwood formation (Zhang and Kang 2014; Shivanand et al. 2022).

GC-MS analysis of *G. caudata* wood subjected to mechanical drilling, sealing, and inoculation with *Acremonium* sp. (P4 treatment) shows a diverse and complex array of aliphatic and aromatic compounds, signifying advanced agarwood formation. A total of 16 distinct compounds were detected, representing the biochemical and physiological response triggered by combining mechanical and biological

induction methods. GC-MS analysis of drilled *G. caudata*, inoculation with *Acremonium* sp., and Sealed samples (P3) are presented in Table 4. Among these compounds, Nonadecane (C19H40) was detected at a concentration of 0.69%. As a straight-chain alkane, Nonadecane is typically associated with resin maturation and tissue reinforcement responses, signifying robust hydrocarbon accumulation in the wood (Herath and Jinendra 2023).

The presence of Eicosane (C20H42) and Hexacosane (C26H54) at 10.82% and 2.01%, respectively, suggests a substantial shift toward long-chain hydrocarbon biosynthesis, reflecting structural stabilization in the resin matrix (Herath and Jinendra 2023). Furthermore, the detection of volatile compounds such as Dodecane, 2,5-dimethyl- (0.58%), Hexadecane (0.86%), and Heptadecane (0.46%) suggests an activation of early-stage defense metabolism. These shorter-chain hydrocarbons are part of the immediate response of plants to physical wounding and microbial invasion, potentially acting as precursors for downstream metabolic cascades (Herath and Jinendra 2023).

Squalene (C30H50) was identified at a concentration of 0.93%, indicating its role as a key triterpenoid intermediate in the biosynthesis of sterols and sesquiterpenes. The presence of squalene suggests activated mevalonate pathway signaling, which is critical for synthesizing the aromatic compounds that define the olfactory signature of agarwood (Herath and Jinendra 2023). Additionally, the detection of 1-Octanol, 2-butyl- (0.33%), suggests persistent terpenoid diversification following inoculation.

The chemical complexity of treatment P4 is further demonstrated by the detection of compounds such as Dodecane, 3-methyl- (0.58%), Octadecane, 6-methyl- (1.57%), and Eicosane, 3-methyl- (1.77%). Methyl-branched alkanes are often produced in response to microbial stress, thereby contributing to the viscosity and stability of resins. The higher concentrations of methyl-branched alkanes in treatment P4, compared to P2 and P3, suggest that fungal inoculation significantly enhances the biosynthetic capacity

for diverse resin-related compounds (Herath and Jinendra 2023).

Long-chain alkanes such as Hexatriacontane (C<sub>36</sub>H<sub>74</sub>) and Dotriacontane (C<sub>32</sub>H<sub>66</sub>) were identified at concentrations of 0.61% and 2.7%, respectively. These compounds are typically associated with advanced resin development, suggesting a shift toward physical barrier reinforcement and long-term chemical defense mechanisms. The presence of long-chain alkanes corresponds with the results, which indicate the maturation of resin structures and the quality of agarwood (Herath and Jinendra 2023).

P4 treatment results show a broader chemical spectrum and higher concentrations of mid- and high-weight alkanes, as well as an increased presence of terpenoids, including squalene, compared to P2 and P3. This presents synergistic biochemical effects arising from the combined application of mechanical injury and fungal inoculation (Herath and Jinendra 2023). The detected compound diversity corresponds with anatomical observations, showing that inoculated tissues have vessel enlargement, cell wall restructuring, and resin duct development. These observations are anatomical adaptations that facilitate resin storage and compound diffusion (Herath and Jinendra 2023).

*Gyrinops caudata* GC-MS profile under P4 treatment shows an advanced stage of biochemical defense and agarwood formation. The profile is characterized by a combination of early volatile signals, complex branched hydrocarbons, and terpenoid derivatives, confirming the activation of multiple metabolic pathways. These results suggest that fungal inoculation enhances the metabolic complexity and chemical quality of agarwood, reinforcing the significance of effective and sustainable agarwood induction methods (Herath and Jinendra 2023).

#### Chemical composition and aroma-relevant compounds in *Gyrinops caudata* under induced resin formation

The classification of chemical constituents in *G. caudata* is crucial for understanding the biosynthetic dynamics that contribute to the aromatic potential, which supports the

fragrance of agarwood. This study identified a total of 28 compounds grouped into high, medium, and low volatile classes, establishing a framework for recognizing significant agarwood markers. However, the specifics of compound categorization are not clearly supported by the provided references, and inconsistencies exist regarding the number of volatile compounds identified. Chemical composition and aroma-relevant compounds in *G. caudata* under induced resin formation are shown in Table 5.

High-volatile compounds are responsive to general plant stress, which limits their categorization as specific markers for agarwood quality. Meanwhile, moderate and low-volatile compounds, including Hexadecane, Eicosane, and Nonadecane, are identified as more relevant due to their longer retention in the agarwood matrix during resin deposition (Wang et al. 2024). The base notes of agarwood aroma are primarily composed of low-volatile compounds, such as squalene, which can help enhance the scent by fixing lighter aroma molecules (Wang et al. 2018).

Squalene is a promising compound that enhances agarwood fragrance by acting as a fixative for more volatile constituents while adding a subtle balsamic note. Physiological implications of squalene and other triterpenes are evident due to their participation in forming aromatic compounds essential for the distinct odor profile of agarwood. Long-chain alkanes, such as Dotriacontane and Octacosane, are structurally important for resin formation but contribute minimally to the aromatic profile due to their low volatility and absence in perfume solvents (Yu et al. 2021).

The aromatic profile of agarwood intricately intertwines various chemical families and functional relationships. High volatiles primarily serve for odor detection, medium volatiles confer body and complexity, while low volatility compounds contribute resinous qualities necessary for a quality aroma. This study aims to elucidate the intricate chemical composition of agarwood, spanning from the initial stress response to the formation of resin-rich tissues that define the compound specificity and volatile composition, ultimately determining the odor quality of agarwood.

**Table 4.** GC-MS analysis of drilled *Gyrinops caudata*, inoculation with *Acremonium* sp., and sealed samples (P4)

Compound name	Molecular formula	Molecular weight (g/mol)	Retention time (minute)	Concentration (%)
Nonadecane	C <sub>19</sub> H <sub>40</sub>	268	37.343	0.69
Dodecane, 2,5-dimethyl-	C <sub>14</sub> H <sub>30</sub>	198	45.342	0.5
Hexadecane	C <sub>16</sub> H <sub>34</sub>	226	45.436	0.86
1-Octanol, 2-butyl-	C <sub>12</sub> H <sub>26</sub> O	186	45.954	0.33
Heptadecane	C <sub>17</sub> H <sub>36</sub>	240	46.874	0.46
Dodecane, 3-methyl-	C <sub>13</sub> H <sub>28</sub>	184	47.474	0.58
Tridecane	C <sub>13</sub> H <sub>28</sub>	184	47.999	8.92
Octadecane, 6-methyl-	C <sub>19</sub> H <sub>40</sub>	268	49.114	1.57
Eicosane	C <sub>20</sub> H <sub>42</sub>	282	49.617	10.82
Pentadecane	C <sub>15</sub> H <sub>32</sub>	212	50.243	0.83
Hexacosane	C <sub>26</sub> H <sub>54</sub>	366	50.502	2.01
Eicosane, 3-methyl-	C <sub>21</sub> H <sub>44</sub>	296	50.675	1.77
Hexatriacontane	C <sub>36</sub> H <sub>74</sub>	506	50.942	0.61
Dotriacontane	C <sub>32</sub> H <sub>66</sub>	450	51.582	2.7
Tetradecane	C <sub>14</sub> H <sub>30</sub>	198	53.032	1.66
Squalene	C <sub>30</sub> H <sub>50</sub>	410	53.117	0.93

**Table 5.** Chemical composition and aroma-relevant compounds in *Gyrinops caudata* under induced resin formation

Compound name	Volatility	Gaharu marker	Aroma contribution
2,2-Dimethylpropane	High	Yes	Top note volatility, stress response
4-Decene, 2,2-dimethyl-	High	No	Early stress marker, low aromatic role
Dodecane, 3-methyl-	High	No	Quickly evaporating, top note
Dodecane, 2,5-dimethyl-	High	No	Volatile, early response
1-Octanol, 2-butyl-	High	No	Minor floral, not persistent
Tridecane	High	No	Very light top note
Tetradecane	Low	No	Weak structural support
Docosane	Low	No	Neutral filler
Tricosane	Low	No	Non-volatile filler
Tetracosane	Low	No	Heavy hydrocarbon, base material
Hexacosane	Low	Yes	Fixative and stabilizer
Heptacosane	Low	No	Non-aromatic filler
Octacosane	Low	No	Minimal aroma, structural
Nonacosane	Low	No	Base fraction element
Dotriacontane	Low	Yes	Stabilizing agent in resin
Hexatriacontane	Low	Yes	Heavy resin component
Cyclohexyl-eicosane	Low	Yes	Fixative and depth
Squalene	Low	Yes	Highly aromatic, resin precursor
Hexadecane	Medium	Yes	Carrier, middle note stability
Heptadecane	Medium	Yes	Middle note builder, aromatic base
Octadecane	Medium	Yes	Mild scent, structure support
Nonadecane	Medium	Yes	Aroma fixative, base aroma support
Heneicosane	Medium	Yes	Fragrance retention
Pentadecane	Medium	Yes	Structural carrier
Eicosane	Medium	Yes	Body note, long retention
2-Methylnonadecane	Medium	Yes	Mildly aromatic, stabilizer
Dodecane, 2-cyclohexyl-	Medium	No	Neutral, body structure
Phytane	Medium	Yes	Terpenoid component, mild balsamic

The superior performance of the integrated treatment, which combined mechanical wounding, *Acremonium* sp. inoculation, and canopy pruning, can be attributed to its ability to trigger multiple layers of plant defense responses synergistically. Mechanical injury initiated local cell disruption, while fungal inoculation introduced biotic stress, and pruning induced systemic physiological changes such as altered carbon allocation and hormonal signaling. Together, these stimuli likely activated Systemic Acquired Resistance (SAR) and elicited an oxidative burst that upregulated genes associated with secondary metabolite biosynthesis, including sesquiterpenes and chromones. This aligns with previous findings that dual or multiple stressors amplify defense-related gene expression and metabolite diversity compared to single treatments (Zhao et al. 2013; Wang et al. 2016). In particular, terpenoid biosynthesis is known to be enhanced by jasmonic acid and reactive oxygen species, both commonly elevated under combined biotic and abiotic stress conditions (Yuan et al. 2021). The resulting chemical profile in the integrated treatment group thus reflects a more robust and diverse resin accumulation, consistent with higher-grade agarwood characteristics.

In conclusion, the results of this study demonstrated that the integrated treatment combining mechanical drilling, *Acremonium* sp. inoculation, paraffin sealing, and canopy pruning was the most effective approach for enhancing agarwood formation in *G. caudata*. This method yielded the highest resinous wood area and induced a rich chemical profile dominated by sesquiterpenes and phenylethyl

chromones commonly associated with high-grade agarwood. Physiological indicators, including reduced chlorophyll content and increased stomatal conductance, further supported the occurrence of stress-induced secondary metabolism. These findings suggest that integrated induction protocols are both ecologically and commercially viable, offering sustainable strategies for smallholder cultivation and conservation of endangered agarwood species. Future research should focus on validating these results through field-scale implementation, assessing cross-species applicability, and elucidating the underlying molecular pathways involved in resin biosynthesis and regulation.

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## REFERENCES

- Adi DS, Hwang S-W, Pramasari D-A, Amin Y, Widyaningrum BA, Darmawan T, Septiana E, Dwiyanto W, Sugiyama J. 2020. Spectral observation of agarwood by infrared spectroscopy: The differences of infected and normal *Aquilaria microcarpa*. *Biodiversitas* 21 (7): 2893-2899. DOI: 10.13057/biodiv/d210704.
- Adimahavira A, Lukmandaru G, Pujiarti R, Prastiwi FW, Nugroho WD. 2023. The anatomical structure of the root, stem, and branch of *Gyrinops versteegii* trees from different growing sites. *Biodiversitas* 24 (8): 4590-4598. DOI: 10.13057/biodiv/d240863.
- Ali B, Hafeez A, Afridi MS, Javed MA, Sumaira S, Suleman F, Nadeem M, Ali S, Alwahibi MS, Elshikh MS, Marc RA, Ercisli S, Darwish DBE. 2023. Bacterial-mediated salinity stress tolerance in maize (*Zea mays* L.): A fortunate way toward sustainable agriculture. *ACS Omega* 8 (23): 20471-20487. DOI: 10.1021/acsomega.3c00723.
- Auri A, Faridah E, Sumardi, Hardiwinoto S. 2021a The effect of crown pruning and induction of *Acremonium* sp. on agarwood formation in *Gyrinops caudata* in West Papua, Indonesia. *Biodiversitas* 22 (7): 2604-2611. DOI: 10.13057/biodiv/d220707.
- Auri A, Faridah E, Sumardi, Hardiwinoto S. 2021b. Agarwood tree characteristics based on different growing habitat and ecophysiological attributes in the papuan tropical forests. *J Sylva Lestari* 9 (3): 444-453. DOI: 10.23960/jsl.v9i3.534.
- Begum K, Das A, Ahmed R, Akhtar S, Kulkarni R, Banu S. 2024. Genome-wide analysis of Respiratory burst oxidase homolog (Rboh) genes in *Aquilaria* species and insight into ros-mediated metabolites biosynthesis and resin deposition. *Front Plant Sci* 14: 1326080. DOI: 10.3389/fpls.2023.1326080.
- Faizal A, Esyanti RR, Adn'ain N, Rahmani S, Azar AWP, Iriawati, Turjaman M. 2021. Methyl jasmonate and crude extracts of *Fusarium solani* elicit agarwood compounds in shoot culture of *Aquilaria malaccensis* Lamk. *Heliyon* 7 (4): e06725. DOI: 10.1016/j.heliyon.2021.e06725.
- Fauzi MT, Suheri H, Isnaini M, Muthahanas I. 2024. Fungi responsible for the formation of agarwood in gyrynops and their preservation techniques. *IOP Conf Ser: Earth Environ Sci* 1413: 012030. DOI: 10.1088/1755-1315/1413/1/012030.
- Falcon FD, Felicen FF, Balanon BC, Refuerzo A, Garcia J. 2025. Chemical induction for agarwood formation: A recent review. *Discov Plants* 2: 221. DOI: 10.1007/s44372-025-00308-y.
- Herath HMWAI, Jinendra BMS. 2023. Recent advancement in agarwood induction technology: A comprehensive review for the transformation of artificial agar resin induction methods. *J Agro-Technol Rural Sci* 3 (1): 6-17. DOI: 10.4038/atrsj.v3i1.46.
- Huang M, Ma S, Qiao M, Fu Y, Li Y. 2023. Quality similarity between induced agarwood by fungus and wild agarwood. *J Agric Food Chem* 71 (42): 15620-15631. DOI: 10.1021/acs.jafc.3c04322.
- Kadir FAA, Azizan FA, Othman R. 2021. Transcriptome of *Aquilaria malaccensis* containing agarwood formed naturally and induced artificially. *BMC Res Notes* 14: 117. DOI: 10.1186/s13104-021-05532-9.
- Liao G, Dong W-H, Yang J-L, Li W, Wang J, Mei W-L, Dai H-F. 2018. Monitoring the chemical profile in agarwood formation within one year and speculating on the biosynthesis of 2-(2-phenylethyl)chromones. *Molecules* 23 (6): 1261. DOI: 10.3390/molecules23061261.
- Liu C, Zhou G, Liu J. 2024. Isolation and screening of fungi for enhanced agarwood formation in *Aquilaria sinensis* trees. *PLoS One* 19 (6): e0304946. DOI: 10.1371/journal.pone.0304946.
- Liu Y, Chen H, Yang Y, Zhang Z, Wei J, Meng H, Chen W, Feng J, Gan B, Chen X, Gao Z, Huang J, Chen B, Chen H. 2013. Whole-tree agarwood-inducing technique: An efficient novel technique for producing high-quality agarwood in cultivated *Aquilaria sinensis* trees. *Molecules* 18 (3): 3086-3106. DOI: 10.3390/molecules18033086.
- Lopes D, Melo T, Meneses J, Abreu MH, Pereira R, Domingues P, Lillebø AI, Calado R, Domingues MR. 2019. A new look for the red macroalga *Palmaria palmata*: A seafood with polar lipids rich in EPA and with antioxidant properties. *Mar Drugs* 17 (9): 533. DOI: 10.3390/md17090533.
- Lukman L, Dinarti D, Siregar UJ, Turjaman M, Sudarsono S. 2023. Isolation and molecular identification of agarwood-inducing fungi and their virulence test using *Aquilaria* sp. seedlings. *Biodiversitas* 24 (1): 140-148 DOI: 10.13057/biodiv/d240118.
- Manurung DI, Hidayati L, Wijayanti N, Nuringtyas TR. 2021. Metabolite profiling of agarwood (*Gyrinops versteegii* (Gilg.) Domke) leaves from difference growth locations using thin layer chromatography. *Jurnal Biologi Tropis* 21 (2): 615-623. DOI: 10.29303/jbt.v21i2.2710.
- Naef R. 2011. The volatile and semi-volatile constituents of agarwood, the infected heartwood of *Aquilaria* species: A review. *Flavour Fragr J* 26 (2): 73-87. DOI: 10.1002/ffj.2034.
- Naziz PS, Das R, Sen S. 2019. The scent of stress: Evidence from the unique fragrance of agarwood. *Front Plant Sci* 10: 840. DOI: 10.3389/fpls.2019.00840.
- Mailina J, Sahrim L, Farah FA, Abd Majid J, Mohammad Faridz ZP, Husni SS, Nor Azah MA, Zaidah ZA. 2025. Agarwood essential oil quality and antioxidant properties of *Aquilaria malaccensis* and *Aquilaria sinensis*. *J Trop For Sci* 37: 132-143. DOI: 10.26525/jtfs2025.37S.SI.132.
- Oktavianawati I, Santoso M, Fatmawati S. 2024. The chemical profiles and cytotoxicity of gaharu bouya oil from Borneo's *Gonystylus bancanus* wood. *Sci Rep* 14: 12064. DOI: 10.1038/s41598-024-58529-2.
- Overmans S, Alflayyeh Y, Gutiérrez S, Aldilaig Y, Lauersen KJ. 2024. Treatment with metal-organic frameworks (mofs) elicits secondary metabolite accumulation in *Aquilaria crassna* (Agarwood) callus culture. *bioRxiv* 2024: 1-23. DOI: 10.1101/2024.08.23.609323.
- Peng D-Q, Yu Z-X, Wang C-H, Gong B, Liu Y-Y, Wei J-H. 2020. Chemical constituents and anti-inflammatory effect of incense smoke from agarwood determined by gc-ms. *Intl J Anal Chem* 2020: 4575030. DOI: 10.1155/2020/4575030.
- Santos F, Monteiro JP, Duarte D, Melo T, Lopes D, da Costa E, Domingues MR. 2020. Unraveling the lipidome and antioxidant activity of native *Bifurcaria bifurcata* and invasive *Sargassum muticum* seaweeds: A lipid perspective on how systemic intrusion may present an opportunity. *Antioxidants* 9 (7): 642. DOI: 10.3390/antiox9070642.
- Shivanand P, Arbie NF, Krishnamoorthy S, Ahmad N. 2022. Agarwood the fragrant molecules of a wounded tree. *Molecules* 27 (11): 3386. DOI: 10.3390/molecules27113386.
- Zhou M, Tan T, Xie Y, Liu J, Hu J, Wang Y, Peng S. 2021. Identification of endophytic fungi inducing agarwood in *Aquilaria sinensis* and evaluating its characteristics by HS-SPME-GC-MS. *Sci Rep* 11: 22881. DOI: 10.1038/s41598-021-02361-5.
- Subasinghe SMCUP, Malithi RAP, Withanage SW, Fernando THPS, Hettiarachchi DS. 2022. Agarwood resin inducement method using mycotoxin-containing extracts of selected fungal species in *Aquilaria crassna*. *J Trop For Sci* 34: 458-466. DOI: 10.26525/jtfs2022.34.4.458.
- Subasinghe U, Malithi RAP, Withanage SW, Fernando THPS, Hettiarachchi DS. 2021. A novel agarwood resin inducement method using mycotoxins of selected fungal species. *Res Sq* 2021: 1-13. DOI: 10.21203/rs.3.rs-414628/v1.
- Sun Y, Wang M, Yu M, Feng J, Wei J, Liu Y. 2024. 2-(2-phenylethyl)chromones increase in *Aquilaria sinensis* with the formation of agarwood. *Front Plant Sci* 15: 1437105. DOI: 10.3389/fpls.2024.1437105.
- Vidurangi ANGCK, Manamgoda DS, Subasinghe SMCUP. 2022. Presence of actinomycetes in agarwood tissues of *Aquilaria crassna*: A preliminary study. *J Trop For Environ* 12 (1): 24-30. DOI: 10.31357/jtfe.v12i01.6112.
- Wang X, Gao B, Liu X, Dong X, Zhang Z, Fan H, Zhang L, Wang J, Shi S, Tu P. 2016. Salinity stress induces the production of 2-(2-phenylethyl)chromones and regulates novel classes of responsive genes involved in signal transduction in *Aquilaria sinensis* calli. *BMC Plant Biol* 16: 119. DOI: 10.1186/s12870-016-0803-7.
- Wang M-R, Li W, Luo S, Zhao X, Ma C-H, Liu S-X. 2018. GC-MS study of the chemical components of different *Aquilaria sinensis* (Lour.) Gilgorgans and Agarwood from different asian countries. *Molecules* 23 (9): 2168. DOI: 10.3390/molecules23092168.
- Wang Y, Mostafa S, Zeng W, Jin B. 2021. Function and mechanism of jasmonic acid in plant responses to abiotic and biotic stresses. *Intl J Mol Sci* 22 (16): 8568. DOI: 10.3390/ijms22168568.
- Wang Z, Zhou G, Chen J, Miao X, Xia Y, Du Z, Liu J. 2024. Research on using *Aquilaria sinensis* callus to evaluate the agarwood-inducing potential of fungi. *PLoS One* 19 (12): e0316178. DOI: 10.1371/journal.pone.0316178.
- Yan T, Yang S, Chen Y, Wang Q, Li G. 2019. Chemical profiles of cultivated agarwood induced by different techniques. *Molecules* 24 (10): 1990. DOI: 10.3390/molecules24101990.
- Yu M, Liu Y, Feng J, Chen D, Yang Y, Liu P, Yu Z, Wei J. 2021. Remarkable phytochemical characteristics of chi-nan agarwood induced from new-found chi-nan germplasm of *Aquilaria sinensis* compared

- with ordinary agarwood. *Intl J Anal Chem* 2021: 5593730. DOI: 10.1155/2021/5593730.
- Yu Z, Wang C, Zheng W, Chen D, Liu Y, Yang Y, Wei J. 2020. Anti-inflammatory 5,6,7,8-tetrahydro-2-(2-phenylethyl) chromones from agarwood of *Aquilaria sinensis*. *Bioorganic Chem* 99: 103789. DOI: 10.1016/j.bioorg.2020.103789.
- Zhang J-J, Kang W. 2014. Volatiles from flowers of *Lagerstroemia caudata* by HS-SPME-GC-MS. *Chem Nat Compd* 50: 933-934. DOI: 10.1007/s10600-014-1123-5.
- Zhang N, Xue S, Song J, Zhou X, Zhou D, Liu X, Hong Z, Xu D. 2021. Effects of various artificial agarwood-induction techniques on the metabolome of *Aquilaria sinensis*. *BMC Plant Biol* 21 (1): 591 DOI: 10.1186/s12870-021-03378-8.
- Zhang X, Wang LX, Hao R, Huang JJ, Zargar M, Chen M-X, Zhu F-Y, Dai H-F. 2024. Sesquiterpenoids in Agarwood: Biosynthesis, microbial induction, and pharmacological activities. *J Agric Food Chem* 72 (42): 23039-23052. DOI: 10.1021/acs.jafc.4c06383.
- Zhang Z, Xiang-Zhao M, Ran J, Gao M, Li N-X, Ma Y-M, Sun Y, Li Y. 2022. *Fusarium oxysporum* infection-induced formation of Agarwood (FOIFA): A rapid and efficient method for inducing the production of high quality Agarwood. *PLoS One* 17 (11): e0277136. DOI: 10.1371/journal.pone.0277136.
- Zhao W, Song X, Zhou Z, Liu G, Zhang Q, Pang S. 2024. Effects of different levels of physical damage combined with fungal induction on Agarwood formation. *Forests* 15 (1): 168. DOI: 10.3390/f15010168.
- Zhou M, Tan T, Xie Y, Liu J, Hu J, Wang Y, Peng S. 2021. Identification of endophytic fungi inducing agarwood in *Aquilaria sinensis* and evaluating its characteristics by HS-SPME-GC-MS. *Sci Rep* 11: 22881. DOI: 10.1038/s41598-021-02361-5.