Secondary Metabolites Characteristics and their Effect on Biological Performance of *Simpur* Wood (*Dillenia sp*.)

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Abstract. This research aimed to assess how secondary compounds found in *Dillenia* wood could enhance the wood's ability to resist white rot fungi (*Trametes versicolor*). The research methodology involved extracting these compounds using acetone and methanol, followed by a fractionation process. The study's findings demonstrated that *Dillenia* wood extracts effectively inhibited the growth of white rot fungi, resulting in a high level of resistance. The major secondary compounds identified in this wood were phenolic compounds. The bioactive compounds' analysis showed these compounds could be found in acetone and methanol extracts.

Keywords: *Dillenia sp*., Secondary Metabolites, White Rot Fungus.

1.0 Introduction

The capacity of white, brown, and soft rot fungi to break down wood cell walls has drawn a great deal of research attention, as have fungi that may infect and colonize wood. This is because these fungi have the potential to cause significant harm to wooden materials that are in use, as well as failure [1]. Fungus infection lowers the wood's quality and durability. Wood-rotting fungi cause significant financial losses in lignocellulosic products; in Finland, they are reported to be around €35,000,000 [2] in Indonesia, they are reported to be as high as Rp17 trillion per year [3]. Fungi that attack wood cause around one-third of the yearly timber harvest (20 billion board feet) to be lost [4].

To optimize the utilization of forest resources, especially wood, there is a need for strategies to prolong the wood's longevity. One approach involves using chemical preservation processes. Unfortunately, the most commonly used preservatives are synthetic chemicals, which have adverse environmental impacts. To address this concern, it is essential to explore more environmentally friendly alternatives, such as preservatives derived from plant extracts [5].

Dillenia sp., a woody plant in the Dilleniaceae family, is locally known as "Simpur" in Indonesia. It grows in tidal marsh environments. *Dillenia* wood falls into the class II–III durability and class II strength levels. This plant's components are all

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used for different things. While the wood bark extract has components that suppress free radicals and also shows resistance to subterranean termites, the stem bark extract has antioxidant-rich phenolic compounds and flavonoids [6]. Flavonoids and polyphenols are among the active components found in *Dillenia* wood extractives that have the ability to prevent the growth of wood-rotting fungus. *Dillenia* leaf extract has also been found effective in controlling the greyback cankerworm, resulting in a mortality rate ranging from 75-80% [7]. Based on these findings, it is important to analyse bioactivity of *Dillenia* wood extract against wood-rotting fungi.

2.0 Literature Review

2.1 Bioactivity of Secondary Metabolites of Wood

To extend the timber's life, one attempt is to provide preservatives into the wood structure. However, the preservatives that are often used are CCA-based chemicals that endanger the environment and human health [8]. The use of natural substances with biocidal properties and their incorporation into the wood structure is an additional strategy for increasing the service life of wood [9, 8]. Numerous plant species with a wide range of chemical defence and survival mechanisms, such as fungicidal and insecticidal components, such as extract of konjac flying powder, tannins [10], flavonoids [11], alkaloids [12], and essential oils derived from plants [13].

Numerous investigations have been carried out to investigate the biological functions of different plants as insecticides, such as botanical insecticides based on rotenone. Since 1850, this bioactive substance has been available; it was extracted from *Derris elliptical* (Wall.) Benth. Rotenone has been found to be an active component in every part of the plant, including the roots, bark, and leaves. Farmers have been using this substance for a long time as a safe insecticide to get rid of pests in plants and vegetables, especially long bean vegetables. In the sphere of fisheries, *Derris elliptical* root extract is used to poison fish, anesthetize fish during freshwater fishing, and kill *Aedes aegypty* larvae and *Aedes sp.* due to its flavonoid concentration.

Some species' natural endurance is mostly attributed to the extractives that build up in the heartwood, some of which slow down the decaying process [14]. These are the non-structural parts of wood, and while some studies have already shown that some extracts contain insecticidal and antifungal qualities, further study has to be done on this subject.

2.2 Wood-Rotting Fungi

Wood is a naturally occurring, renewable, extremely adaptable, and high-performing material that has been widely utilized by humans since the beginning of time. But because of its structure and chemical makeup, wood is susceptible to biodeterioration, with fungus being the primary degrader. Fungi's ability to biodegrade wood is acknowledged as one of the biggest threats to wooden constructions and forest

management [15, 16]. Three categories of fungi that degrade wood are traditionally identified based on their patterns of degradation: brown rot, white rot, and soft rot [15]. They all weaken the hardwood cell wall's structural polymers, which causes the wood to lose its strength. Moreover, blue stain and mould can harm wood. Their activity results in wood discoloration, which negatively affects the aesthetic value of wood even though it does not significantly impact structural integrity [16].

As the only living species that can break down wood to its basic components, wood decay fungi are vital and significant among the related organisms. The ecosystem as a whole will collapse in their absence since material cycles and energy fluxes are impossible to achieve [17]. As a result, among the most significant decomposers in the forest environment are fungi that cause wood rot. The term "wood decay fungi" refers to a general category of fungus, including those that grow on different types of wood substrates, rather than a specific species or taxonomic classification.

3.0 Methodology

3.1 Extraction of *Dillenia* Wood

Heartwood mill (40-60 mesh) of *V. cofassus* was prepared and extracted according to the procedure reported previously [18] with slight modification. In this study, the milled wood that was used instead of small wood-pieces was firstly extracted with acetone and then the residue was extracted again with methanol until the extract solution became colourless. These acetone and methanol extracts were then successively fractionated into n-hexane, ethyl acetate, and water to give their soluble fractions.

3.2 Cultivation of Wood-Decaying Fungi

Wood-decaying fungi are cultivated using Malt Extract Agar (MEA) as the growth medium, which is prepared by mixing 39 grams of Malt Extract with one litre of distilled water or aquades in an Erlenmeyer flask. The mixture is heated on a hotplate, homogenized, and then sterilized in an autoclave at 120 ± 2°C for 15 minutes under a pressure of 1.5 atm. After autoclaving, the medium is poured into petri dishes, allowed to cool, and sealed with plastic wrap. The fungi are then inoculated into the medium, and the petri dishes are incubated until the fungi exhibit uniform growth.

3.3 Efficacy of *Dillenia* Wood Extract Against Wood-Decaying Fungi

Fungal bioassay was conducted using the Malt Extract Agar (MEA) medium containing 50 and 100 ppm of samples (the extracts and the fractions) in a Petri dish [19]. Triplicate media for each amount of the samples were prepared. The MEA powder in distilled water (39g/L) was autoclaved, and to this warm medium (40-50°C) was added a solution of each of the samples in methanol, and the whole was shaken. Three

parts from the resulting mixture were transferred equally into three Petri dishes, respectively, and the mycelium disk was placed at the centre of the medium. MEA plates containing methanol without the samples were used as a control. The media were incubated at 23°C. When the mycelium of fungi on the control medium reached the edge of the Petri dish, the antifungal activity (AFA) index (%) was calculated as follows: AFA index (%) = (1-Da/Db) x 100, where Da: diameter of mycelium colony growth with the samples (cm), Db: diameter of mycelium colony growth in the control (cm). Based on the AFA value, the activity of each fraction was classified into the following category levels [20]. AFA ≥ 75% (very strong), 75%≤ AFA < 50% (strong), 50% ≤AFA < 25% (moderate), 25% ≤ AFA < 0 (weak), and 0 (not active).

3.4 Determine of polyphenol and flavonoid content

To determine polyphenol content, 0.1 g of methanol extract is dissolved in 100 ml of methanol. A 0.05 ml sample is placed in a 10 ml volumetric flask. Then, 2.5 ml of Folin-Denis reagent and 2 ml of NaOH are added, and distilled water fills the flask to the 10 ml mark. A blank solution is prepared in a similar manner. The absorbance is measured at 756 nm using a UV-Vis spectrophotometer. Total polyphenol content is calculated via a calibration curve with gallic acid, following the formula by Sayyidah et al. [21].

$$Pf=\frac{c \times V \times f \times 10^{-3}}{m-(KA \times m)} \times 100 \quad (1)$$

Where:
Pf: Total polyphenol content (%) c: Concentration of gallic acid (ppm) V: Total extract volume (ml) f: Dilution factor m: Sample weight (g) KA: Moisture content

To determine flavonoid content, 0.1 g of methanol extract is dissolved in 100 ml of methanol. A 1.0 ml sample is placed in a 10 ml volumetric flask, and 2 ml of CH3COONa and $AlCl_3$ solutions are added. Ethanol is used to fill the flask to the 10 ml mark. A blank solution is created similarly. The absorbance is measured at 434.2 nm using a UV-Vis spectrophotometer. Total flavonoid content is calculated using a calibration curve with quercetin, following Azizah et al. [22] formula.

$$F=\frac{c \times V \times f \times 10^{-6}}{m} \times 100 \quad (2)$$

Where:
F: Total flavonoid content (%)
c: Quercetin equivalence (ppm)
V: Total extract volume (ml)
f: Dilution factor

m: Sample weight (g)

3.5. Data Analysis

In the fungal testing, the data analysis primarily consists of a comparative assessment, which involves comparing the antifungal index between the treatment samples and the control group. Furthermore, the characteristics of metabolites secondary of the *Dillenia* wood are subject to descriptive analysis, particularly focusing on the percentages of polyphenol and flavonoid content.

4.0. Results and Discussions

4.1 Extraction Results

Generally, organic solvents can be distinguished into two categories, a polar solvent and a non-polar solvent. Based on the extraction performed, acetone solvent has a greater yield than the methanol solvent, and the use of solvent types with different levels of polarity greatly affects the result of extraction.

Table 1. Result of *Dillenia* wood powder extraction.

No	Results	Acetone	Methanol
1.	Sample Weight (g)	114,35	
2.	Extract Weight (g)	9,5	3,1
3.	Fractionation Yield (%)	8,3	2,71
4.	Colour	Dark red	Brownish-Red
5.	Form	Powder	Powder

This variance is influenced by the different polarities of solvents. Acetone, with a lower polarity (constant value of 21), is more effective in extracting *Dillenia* wood extract due to its similarity in polarity with the wood's compounds, suggesting the non-polar properties of *Dillenia* wood extract. The extractives comprise both inorganic and organic components. Ash was used to quantify the inorganic components, and its content was limited to 1% of the dry weight of the wood. The incredibly vast number of individual lipophilic and hydrophilic chemicals that make up the organic components typically make up less than 10% of the dry weight of the wood, though this can vary from traces to 40% [23].

4.2 Fractionation Results

The results from the fractionation of acetone and methanol extracts of *Dillenia* wood indicate that a higher quantity of non-polar compounds was extracted compared to semi-polar and polar compounds. Detailed information is presented in Table 2.

Table 2. Result of fractionation of acetone and methanol extracts.

No.	Results	Acetone			Methanol		
		n-Hexane	Ethyl Acetate	Water	n-Hexane	Ethyl Acetate	Water
1.	Sample Weight (g)		1			1	
2.	Extract Weight (g)	0,31	0,04	0,26	0,24	0,23	0,23
3.	Fractionation Yield (%)	31	4	26	24	23	23
4.	Color	Dark red	Brown	Dark brown	Brown	Dark red	Dark red
5.	Form	Powder	Paste	Powder	Powder	Powder	Powder

Variations in the fractionation process are shown in Table 2, which also shows the existence of distinct chemicals extracted by solvents with varied polarity. Although ethyl acetate and water have higher polarities and produce distinct molecules, n-hexane, a non-polar solvent, is effective in extracting non-polar components. According to the results, *Dillenia* wood extract contains a significant concentration of non-polar compounds, with n-hexane producing the maximum fractionation. Wax, lipids, essential oils, flavonoids, phenols, steroids, triterpenoids, alkaloids, and astegonin are examples of components that fall under this category.

4.3. Antifungal Activity

The evaluation against white rot fungi continued until the entire surface of the control media was covered by fungal mycelium, indicating the completion of the testing process. Subsequently, the Antifungal Activity (AFA) values were calculated. These AFA values for each sample, treated with *Dillenia* wood extract and its fractions, are presented in Table 3.

Table 3. Classification of the antifungal activity index of *Dillenia* wood extractives.

Concentration	Extract and Fraction	Antifungal Activity (%)	Category
50 ppm	Acetone	100	Very Strong
	n-Hexane	100	Very Strong
	Ethyl Acetate	100	Very Strong
	Water	100	Very Strong
	Methanol	100	Very Strong
	n-Hexane	100	Very Strong

	Ethyl Acetate	100	Very Strong
	Water	100	Very Strong
100 ppm	Acetone	100	Very Strong
	n-Hexane	100	Very Strong
	Ethyl Acetate	100	Very Strong
	Water	100	Very Strong
	Methanol	100	Very Strong
	n-Hexane	100	Very Strong
	Ethyl Acetate	100	Very Strong
	Water	100	Very Strong

Table 3 demonstrates the efficacy of all extracts and their fractions against wood rotting fungi, which have exhibit very strong with AFA value of 100%. In comparison, Syahidah's [24] study reported that bitti wood (*Vitex cofassus*) displayed antifungal index values ranging from 30.3% to 54.4% against white rot fungi (*T. versicolor*). Therefore, it's mean that *Dillenia* wood exhibit high resistance against *T. versicolor* compared to bitti wood. Flavonoids will regulate fungal cell proteins, interfere with the lipid layer, and result in cell damage to the fungus. Polyphenols are fungistatic compounds, which means they can inhibit the growth of fungi without turning them off. The mechanism of action of polyphenols in the inhibition of fungal growth is almost the same as that of flavonoids, which regulate proteins in fungal cells that cause fragility in the cell walls so that it is easy to penetrate another bioactive compound. If the denatured protein is an enzyme protein, then the enzymes in the mushrooms will not work which will affect the disturbance of metabolism and the process of nutrient absorption [25-27].

4.4. Bioactive Compounds of Dillenia Wood

The analysis results show that in the acetone extract of Simpur wood, there are 46 compounds that have been successfully extracted, as shown in Table 4 below. The compounds obtained from the acetone extract are mostly aromatic compounds, acyl ester derivatives, and terpenes. Tannin is not found among those compounds.

Table 4. Acetone extract of *Dillenia* wood.

Retention time (mins.)	Compound	Relative abundance (%)
3.036	4-hydroxy-4-methyl-2-pentanon	5.76
4.068	1,3,5-trimethyl-benzene	0.20
4.621	2,5-dimethyl-, formate,(Z)-3-Hexen-1-ol	0.10
5.425	4-ethylL-1,2-dimethyl-benzene	0.12
5.555	Undecane	0.15

5.717	Linalool	0.50
5.940	(E)-2,3-Epoxycarane	0.31
6.003	1,2,4,5-tetramethyl-benzene	0.28
6.167	3,5,5-trimethyl-2-cyclohexen-1-one	0.36
6.452	2,6-Dimethyl-6-nitro-2-hepten-4-one	1.62
6.781	phenylmethyl acetate	0.80
8.887	Undecanal	0.27
9.309	1,2,3-Propanetriol	1.91
10.036	1-Tridecene	0.22
10.307	4,4'-methylenebis[2,6-dimethyl-phenol	2.48
10.756	α-Guaiene	0.11
11.364	trans-β-ionone	0.26
11.625	1,2,4-benzenetriol	0.81
11.683	1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.α,7. α,8a.β)]-azulene	0.51
11.734	2,4-bis(1,1-dimethylethyl)-phenol	0.83
11.842	Methyl dodecanoate	0.99
11.988	Lilial	1.76
12.265	α-(trichloromethyl)-benzenemethyl acetate	0.65
12.710	Ethyl dodecanoate	4.62
12.768	Diethyl phthalate	7.32
14.770	α-Hexyl-cinnamaldehide	2.08
15.133	2-(Phenylmethylene)-octanal	0.13
15.208	Ethyl tetradecanoate	4.18
16.180	4,6,6,7,8,8-Hexamethyl-1,3,4,6,7,8-hexahydrocyclopenta[G]isochromene	0.18
16.335	bis(2-Methylpropyl) 1,2-benzenedicarboxylic acid	17.19
17.422	Methyl Hexadecanoate	1.06
18.176	Dibutyl phthalate	0,92
18.401	9-Octadecenoic acid	0.20
18.900	Ethyl hexadecanoate	17.74
21.315	Methyl 9,12-Octadecadienoate	0.23
21.472	Methyl 9-Octadecenoate	0.82
22.077	Methyl stearate	0.23
22.827	Ethyl linoleate	1.92
22.974	Ethyl oleate	10.43
23.570	Ethyl octadecanoate	2.17
27.663	1-Hexacosene	0.18
29.958	Methyl cassamate	0.17
30.155	1,2-Benzenedicarboxylic Acid	3.68
33.630	bis(2-Ethylhexyl) 1,4-benzenedicarboxylate	1.57
34.400	1-Eicosene	0.12
34.653	Squalene	0.23

The analysis results show that in the methanol extract of Simpur wood, there are 41 compounds that have been successfully extracted, as shown in Table 5 below. Among those compounds we can find alkaloids, acyl ester, sterols, and flavonol. Phenolic compounds can be found in the methanol extract in the form of flavonol. However, tannins are still not to be found probably because the high polarity of tannins, so it might can be found in Simpur wood water extract.

Table 5. Methanol extract of Dillenia wood.

Retention time (mins.)	Compound	Relative abundance (%)
3.136	1-Acetyl-16-methoxy-aspidospermidin-17-ol	7.15
10.304	bis(Trimethylsilyl)monomethyl phosphoric acid	0.56
14.010	2,6,10,14-tetramethyl-hexadecane	0.81
16.331	bis(2-methylpropyl) 1,2-Benzenedicarboxylate	0.73
17.413	Methyl hexadecanoate	2.44
18.340	n-Hexadecanoic acid	4.89
18.616	Hexadecamethyl-heptasiloxane	1.88
18.885	Ethyl hexadecanoate	2.42
19.593	1-Methylethyl hexadecanoate	11.86
21.459	Methyl 9-Octadecenoate	1.35
22.063	Methyl stearate	0.65
22.963	Ethyl Oleate	0.57
28.583	1,3,5-triphenyl-cyclohexane	0.86
28.825	2,2,7,7-tetramethyl-cycloheptanone imine	0.74
28.992	(3.β.,24S)-stigmast-5-en-3-ol	4.16
29.175	1,2,5,9,9-Pentamethyl-spiro(3.5)non-5-en-2-ol	1.87
29.317	(1S,2R,3E,7E,11E)-2,16,19-Trihydroxycembra-3,7,11,15-tetraene	0.68
29.375	Dodecanoyl chloride	0.82
29.425	3.α.,4. α.,9. β.,11-Diepoxymuurolan-10-ol	1.77
29.542	Villosin	3.66
29.850	Pregn-4-ene-3,6-dione	0.90
30.146	1,2-Benzenedicarboxylic acid	2.01
32.617	7,3',4'-Trimethoxy quercetin	0.98
33.342	(11S)-4.xi.-Germacr-9-en-12-oic acid	0.77
33.414	Methyl 2-chloro-eicosanoate	0.60
33.625	bis(2-ethylhexyl) 1,4-benzenedicarboxylic acid	1.24
34.050	1,1,3,3-Tetraallyl-1,3-disilacyclobutane	0.67
34.332	(1S,2E,4E,7E,11E)-10-Oxocembra-2,4,7,11-tetraene	0.48
34.400	Propyl oleate	0.87

34.640	5,9,13,17-Tetramethyl 4,8,12,16-octadecatetraenoic acid	0.97
34.783	N-Butylboronate methyl 9,10-Dihidroxy-Stearate	1.65
35.364	di(2,2-Dichloroethyl) sebacic acid	0.68
36.015	Octahydro-1-(2-octyldecyl)-pentalene	0.76
36.461	2-Ethylhexyl tridec-2-yn-1-yl Fumarate	0.52
37.425	Liguloxide	0.44
39.242	(3. β.)-Stigmasta-5,22-dien-3-ol acetate	0.62
39.685	β-Sitosterol acetate	1.89
40.023	3,5-Diacetoxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)heptane	8.49
40.648	1,5,9,9-Tetramethyl-2-oxatricyclo[6.4.0.0(4,8)]dodecane	0.73
41.524	2-Myristynoyl-glycinamide	1.44
42.304	1,5,9,9-Tetramethyl-2-oxatricyclo[6.4.0.0(4,8)]dodecane	0.70
43.291	Tris(2,4-di-tert-butylphenyl) phosphate	16.47

5.0 Conclusion & Recommendations

Based on the research findings, it can be concluded that extract of Dillenia wood tend to be non-polar and perform a high activity against the white rot fungus (*Trametes versicolor*). This is probably due to the high content flavonoids and polyphenols compounds in the secondary metabolite of wood Dillenia. For future works, we recommend characterize Simpur wood water extract to obtain more tannins.

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Paper Contribution to Related Field of Study. This paper contributes to the development of research in the field of the utilization of chemical components of very extensive forest products and still requires further research to be used by humans.

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