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### Review Article

## A Review on the Potency of *Melaleuca leucadendron* Leaves Solid Waste in Wood Preservation and Its In Silico Prediction upon Biological Activities

# Christine Patramurti<sup>1</sup>, Radjali Amin<sup>1</sup>, Christofori M. R. R. Nastiti<sup>1</sup>, and Maywan Hariono<sup>1</sup>

<sup>1</sup>Faculty of Pharmacy, Sanata Dharma University, Sleman, Daerah Istimewa, Yogyakarta 55282, Indonesia <sup>2</sup>Post-Graduate School, Yogyakarta Institute of Technology, Bantul, Daerah Istimewa, Yogyakarta 55198, Indonesia

Correspondence should be addressed to Maywan Hariono; mhariono@usd.ac.id

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The essential oils from cajuput leaves (*Melaleuca leucadendron*) have been well-known and applied, especially in healthcare management. However, the utilization of the leaves solid waste has not been explored and reported in detail. In this review, we elaborate on the cajuput leaves starting from the plant description and leaf morphology, chemical composition, biological activities, wood decomposing organism, and an in silico prediction upon its molecular mechanism. Based on the in silico prediction, compounds such as guaiol, lupene, and 1, 8-cineole have the potential to be antifungal and insecticide that associates with the cajuput potency as a wood preservative agent.

#### 1. Introduction

The leaves and small branches distillation process of cajuput (*Melaleuca leucadendron* (L.) L.) produces essential oils with eucalyptol (cineole) as the main chemical substance (50–65%) [1]. Eucalyptol (1, 3, 3-trimethyl-2-oxabicyclo [2.2.2]octane) is classified as the oxygenized monoterpene hydrocarbon group. This compound plays an important role in its antibacterial activity against Gram-positive and Gramnegative bacteria. The biological activities were also reported against fungus and insects which could be due to that one of the major eucalyptol compounds borne a chemical structure as 1, 8-cineole (Figure 1) [1].

Besides the essential oils, the distillation process of cajuput produces solid residue (waste), i.e., the cajuput leaves mixed with small and big branches. The leaves solid waste still contains the essential oil which composes chemicals similar to its fresh leaves when they were distilled. In Maluku (Indonesia), the waste was produced in approximately 51 million tons per year up to 2013 [2], whereas in West Java, the waste production was up to 8.3 million tons per year [3]. It is only about 14% of waste was estimated to be directly utilized by the cajuput distillation factory [4].

The research reported that solid cajuput waste can be used as activated charcoal [5]. The waste can be further processed as the biopellet and briquette charcoals [6]. The calorie value of cajuput briquette charcoal is much higher than the common briquette charcoals [2] that can reach up to 1.5 times magnitude [7, 8]. This solid waste also contains volatile gases with a quantity of up to 47-68% [5, 7]. This waste was also utilized as fuel such as in a steam boiler [9] and as a food substitute for cow cattle on the farm [10]. A study by Rahmawati et al. reported that cajuput waste can be processed as an organic soil fertilizer [4]; however, the process of decomposition was slow triggering several studies to investigate the environmental factors accelerating this waste decomposition process [11]. Interestingly, no study has been carried out to investigate the chemical substances in cajuput solid waste which might slow down the decomposition process.





FIGURE 1: The chemical structure of 1, 8-cineole.

The 1, 8-cineole which is one of the main components of eucalyptol [1] is predicted as the chemical which inhibits the decomposition due to its activities as an antibacterial, antifungal, and insecticide agent. To date, no study identifies the concentration of 1, 8-cineole in the cajuput leaves solid waste and the probability of other compounds supporting the role in the decomposition inhibition. It is estimated that if these chemicals can be well isolated, they may be further utilized in the wooden furniture preservation, thereby increasing the economic values of the solid cajuput waste. This review article focuses on the progress studying the chemical constituent identification and the biological activities of cajuput leaves. Subsequently, the in silico prediction on how the chemicals in cajuput leaves solid waste interact with the protein expressed by wood decomposing microorganisms is also reviewed.

#### 2. Plants Description and Leaf Morphology

The genus of Melaleuca (Myrtaceae family) covers at least 230 species in the world, mostly found in Tasmania (Australia), Indonesia, Papua New Guinea, Tropical America, and South Asia [12, 13]. The main genera utilized for the production of commercial essential oils and aromatherapy are Melaleuca alternifolia (Maiden and Betche) Cheel (tea tree oil), Melaleuca cajuputi Powell (cajuput oil), and Melaleuca quinquenervia (Cav.) S. T. Blake (niaouli oil). Other well-known genera are Melaleuca leucadendron, Melaleuca viridiflora, Melaleuca acacioides, Melaleuca alsophila, Melaleuca bracteata, and Melaleuca argentea [14, 15]. The trees of Melaleuca leucadendron are approximately 40 m high, which is grown in northern Australia, the south coast of New-Guinea, and the east coasts of Indonesia [12]. The morphology of Melaleuca leucadendron leaves is a blade narrowly ovate, very narrowly ovate, rarely narrowly elliptic, or very narrowly elliptic (often falcate to subfalcate). The leaves are 3.5-16 times longer compared to the width; petals are with elliptic oil glands (occasionally long elliptic glands form an apparently linear gland) [16]. The tree of cajuput and its leaves are presented in Figure 2. A number of protocols have been applied to standardize the components of the leaves and the extract including phytochemical screening, thin layer chromatography profile, organoleptic evaluation, histological and microscopic techniques of leaves, powder microscopic, physicochemical parameters, quantitative microscopy, and evaluation of volatile oil [17-23].

#### 3. Chemical Compositions

Most of the chemical composition of cajuput was found from its leaves. The (E, S)-nerolidol and related alcohols such as farnesol and geraniol were found from the leaves of *Melaleuca leucadendron* which was used in feeding deterrent of the gypsy moth larvae [24]. The high content of two phenylpropanoid chemotypes was furthered isolated from the Melaleuca leaves, which were methyl eugenol (99%) and methyl isoeugenol (88%; both in *Z* and *E* configuration). On the other hand, small amounts of trans- $\beta$ -ocimene and linalool were also detected [25]. The chemical structure of eugenol, isoeugenol, trans- $\beta$ -ocimene, and linalool is illustrated in Figures 3(a)–3(d), respectively.

By distillation, the fresh leaves of cajuput were producing essential oils containing *a*-pinene,  $\beta$ -pinene,  $\beta$ -myrcene, limonene,  $\gamma$ -terpinene, 1, 8-cineole, *p*-cymene, terpinolene, benzaldehyde, linalool, trans-pinene hydrate, terpin-4-ol,  $\gamma$ -terpineol,  $\alpha$ -terpineol, 1-tetradecene, ledol, valencene, eugenol,  $\alpha$ -eudesmol, and  $\beta$ -eudesmol as the main constituents (64%) [15]. The similar distillation process was carried out to hydrodistill essential oil from cajuput leaves observing the other major constituents such as  $\beta$ -eudesmol (15.8%),  $\alpha$ -eudesmol (11.3%), viridiflorol (8.8%), and guaiol (9.0%) (Figures 3(e)–3(h), respectively) [26].

A study by Yoshida et al. on the chemical identification of cajuput dried fruits indicated a new hydrolyzable tannin and other polyphenols as the secondary metabolite being deposited. They were 1, 2-di-O-galloyl-3-O-digalloyl-4, 6-O-(S)-hexahydroxydiphenoyl-3-I $\beta$ -glucose (Figure 4(a)) and nine known hydrolyzable tannins. Commonly known stilbene glycoside and triterpene were also identified [27].

A comprehensive study was successfully performed to identify further triterpene from the leaves and heartwood of cajuput. A novel lupene-type nortriterpene (28-norlup-20(29)ene-3 $\beta$ , 17 $\beta$ -diol) as well as 13 other known compounds were characterized. The other 13 compounds were (2E, 6E)-farnesol, phytol, squalene, alloaromadendrene, ledene, palustrol, viridiflorol, ledol, betulinaldehyde, betulinic acid,  $3\beta$ -acetyl-lup-20(29)-en-28-oic acid, 3-oxolup-20(29)-en-28-oic acid, and platanic acid [28]. Further novel triterpenes were characterized, i.e.,  $3\beta$ -cis-coumaroyloxy- $2\alpha$ -hydroxyursa-12, 20(30)-dien-28oic acid, and cis- and trans- $3\beta$ -caffeoyloxy- $2\alpha$ -hydroxyurs-12en-28-oic acids from the leaves as well [29]. Research also successfully identified three new triterpenes from the heartwood of cajuput [30]. They are 23-trans-p-coumaroyloxy- $2\alpha$ , 3β-dihydroxyolean-12-en-28-oic acid, 3β-trans-caffeoyloxy- $2\alpha$ , 23-dihydroxyolean-12-en-28-oic acid, and its isomer 3pcis-caffeoyloxy-2a, 23-dihydroxyolean-12-en-28-oic acid [31]. The general structure of lupene is shown in Figure 4(b).

Having isolated from the heartwood, four new triterpenes were characterized including eupha-7, 24-diene-3 $\alpha$ , 22 $\alpha$ -diol, 20-taraxastene-3R, 28-diol, 3R-hydroxy-13(18)oleanene-27, 28-dioic acid, and 3R, 27-dihydroxy-28, 20 $\alpha$ taraxastanolide. Besides, Lee also identified four novel  $\beta$ -triketone flavanones from the leaves. The compounds were then named as leucadenone A to leucadenone D [32].



FIGURE 2: The habitus of (a) tree of cajuput and (b) its leaves. The photos were taken in Sendang Mole, Playen, Gunung Kidul, Daerah Istimewa Yogyakarta.



FIGURE 3: The chemical structure of (a) eugenol, (b) isoeugenol, (c) trans- $\beta$ -ocimene, (d) linalool (e)  $\beta$ -eudesmol, (f)  $\alpha$ -eudesmol, (g) viridiflorol, and (h) guaiol which are benefit for the antibacterial effect and are the components of hydrodistilled essential oil from cajuput leaves.

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FIGURE 4: The chemical structure of (a) 1, 2-di-O-galloyl-3-O-digalloyl-4, 6-O-(S)-hexahydroxydiphenoyl-3-I $\beta$ -glucose, the chemical composition of cajuput fruits, and (b) the general structure of lupene which are the chemical composition of the leaves.

TABLE 1: Identification and quantification of the compounds in cajuput (Melaleuca leucadendron) (reported in 2011-2020).

No.	Compounds	Part	% or mg	References
1	<i>α</i> -Pinene	Leaves	0.22-0.68	[33]
2	α-Thujene	Leaves	1.29-4.16	[33]
3	β-Pinene	Leaves	0.79-2.90	[33]
4	$\beta$ -Myrcene	Leaves	0.31-0.95	[33]
5	Carene	Leaves	0.29-1.18	[33]
6	D (+)-limonene	Leaves	4.45-8.85	[33]
7	γ-Terpinene	Leaves	1.82-6.72	[33]
8	Terpinolene	Leaves	0.67-3.62	[33]
9	1,8-Cineole	Leaves	44-60	[33]
10	Linalool	Leaves	0.00-0.42	[33]
11	Terpinene-4-ol	Leaves	0.63-0.97	[33]
12	Ôcimenol	Leaves	0.09-0.20	[33]
13	α-Terpineol	Leaves	5.93-12.45	[33]
14	y-Terpineol	Leaves	0.36-2.06	[33]
15	Cedrene	Leaves	0.00-0.61	[33]
16	$\beta$ -Caryophyllene	Leaves	3.78-7.64	[33]
17	Humulene	Leaves	0.53-0.88	[33]
18	$\beta$ -Eudesmene	Leaves	0.98-3.51	[33]
19	Patchoulene	Leaves	0.82-4.37	[33]
20	Germacrene D	Leaves	0.17-0.60	[33]
21	Aromadendren	Leaves	0.00-0.27	[33]
22	Globulol	Leaves	2.70-3.60	[33]
23	Viridiflorol	Leaves	0.00-0.36	[33]
24	Eugenol	Leaves	2.68-4.85	[33]
25	2-Pentanone	Leaves	5.0 mg	[33]
26	Melachromone	Leaves	2.4 mg	[34]
27	Parietin	Leaves	33.2 mg	[34]
28	5-Hydroxy-7-methoxy-2, 6, 8-trimethylchromone	Leaves	11.7 mg	[34]
29	Eugenitin	Leaves	10.2 mg	[34]
30	2, 5-Dihydroxy-7-methoxy-2, 6-dimethylchromanone	Leaves		[34]
31	2, 5-Dihydroxy-7-methoxy-2, 8-dimethylchromanone	Leaves	2.4 mg	[34]
32	Noreugenin	Leaves	8.3 mg	[34]
33	5, 7-Dihydroxy-2, 6, 8-trimethylchromone	Leaves	4.8 mg	[34]
34	Methyl 2-acetyl-3, 5-dihydroxyphenylacetate	Leaves	14.4 mg	[34]
35	Quercitrin	Leaves	3.0 mg	[34]
36	$\beta$ -Sitosterol	Leaves	8.0 mg	[34]
37	5-Hydroxy-7, 40-dimethoxy-6, 8-dimethylflavone	Leaves	12 mg	[34]
38	3, 3', 4-Tri-O-methylellagic acid	Leaves	0.04	[34]
39	Camphene	Leaves	0.14	[35]

No.	Compounds	Part	% or mg	References
40	<i>L</i> -limonene	Leaves	0.04	[35]
41	Rose oxide	Leaves	0.13	[35]
42	Isopulegol	Leaves	0.24	[35]
43	Neral	Leaves	0.13	[35]
44	Geraniol	Leaves	0.31	[35]
45	Geranial	Leaves	0.14	[35]
46	A-terpenyl acetate	Leaves	0.23	[35]
47	Δ-Elemene	Leaves	0.37	[35]
48	α-Ylangene	Leaves	0.36	[35]
49	α-Cubebene	Leaves	1.01	[35]
50	$\beta$ -Elemene	Leaves	0.16	[35]
51	α-Gurjunene	Leaves	0.55	[35]
52	Linalyl acetate	Twigs	0.11	[36, 37]
53	Bornyl acetate	Leaves	0.60	[36, 37]
54	$\beta$ -Bourbonene	Flowers	2.67	[36, 37]
54	$(E)$ - $\beta$ -Farnesene	Flowers	0.10	[36, 37]
55	(Z)-Nerolidol	Twigs	90.85	[36, 37]
56	(E)-Nerolidol	Leaves	0.20	[36, 37]
57	Epi-α-cadinol	Leaves	0.79	[36, 37]
58	$\hat{\beta}$ -Bisabolol	Flowers	0.25	[36, 37]
59	(Z)-Nerolidyl acetate	Twigs	0.25	[36, 37]
60	Palustrol	Leaves	1.86	[36, 37]
61	Ledol	Leaves	0.87	[36, 37]
62	Bulnesol	Leaves	0.21	[36, 37]
63	Alloaromadendrene	Leaves	NR	[36, 37]
64	Melaleucadines A	Branches and leaves	NR	[38]
65	Melaleucadines B	Branches and leaves	0.8	[38]
66	3-Allyl-2-methoxyphenol	Leaves	0.8	[39]
67	(E)-Methyl cinnamate	Leaves	95.4	[39]
68	Eugenol methyl ether	Leaves	NR	[39]
69	(E)-3, 7-Dimethylocta-2, 6-dienal	Leaves	NR	[40]
70	1, 1, 4, 7-Tetramethyl-1a, 2, 3, 4, 4a, 5, 6, 7b-octahydro-	Leaves	NR	[40]
71	1H-cyclopropa[e]azulene	Fruits	NR	[41]
72	(+)-Leumelaleucol A	Fruits	NR	[41]
73	(–)-Leumelaleucol A	Fruits	NR	[41]
74	Leumelaleucol B	Fruits	NR	[41]
75	Leumelaleucol C	Fruits	NR	[41]
76	Leumelaleucol D	Fruits	NR	[41]
77	Leumelaleucol E	Fruits	NR	[41]
78	Leumelaleucol F	Fruits	NR	[41]
79	Leumelaleucol G	Fruits	NR	[41]
80	Leumelaleucol H	Fruits	NR	[41]
81	Leumelaleucol I	Fruits	NR	[41]
82	Leumelaleucol J	Fruits	NR	[41]

TABLE 1: Continued.

NR, not reported.

The investigation of the chemical component in the cajuput has been highly developed in a decade. Novel and known compounds were reported enriching the database of compounds from cajuput (Table 1), which were potential to be developed as medicine, cosmetics, aromatherapy, and fiber technology.

#### 4. Biological Activities

The preservative effect of cajuput leaves was introduced due to its chemical composition to eradicate microorganisms. Biological activities of cajuput against some microbes including bacteria, fungi, virus, and insects are summarized below. 4.1. Antibacterial. Cajuput oil, which was hydrodistilled from the leaves, was tested against the methicillin-resistant *Staphylococcus aureus* (MRSA) colony [42]. The minimum bactericidal concentration (MBC) and the minimum inhibition concentration (MIC) were found to be 0.2% and 0.4%, respectively. This suggested that the leaves oil was able to inhibit the resistant bacteria growth in very low concentrations. This finding could be the potential to overcome bacterial resistance which has currently become an urgent public health issue. Furthermore, the antibacterial test against MRSA was also carried out on the ethanol extract of cajuput leaves. Maceration was used to extract the bactericidal constituents from the leaves, and the extract was tested onto the bacteria using a good well diffusion method. The results demonstrated that ethanol extract was able to inhibit the growth of bacteria. The concentrations of 50%, 60%, 70%, 80%, 90%, and 100% showed an inhibition zone of 17.2 mm, 18.1 mm, 19.1 mm, 19.4 mm, 19.7 mm, and 20.1 mm, respectively [43]. The ethanol extract of cajuput oil was also previously inhibiting *Escherichia coli* bacterial growth with an MIC of  $64 \mu g/mL$  [44].

Cajuput essential oil from leaves demonstrated appreciable activity against *Enterobacter aerogenes* with inhibition zone 13.7–16.2 mm [39]. *Enterobacter aerogenes* is the Gram-negative bacteria of the Enterobacteriaceae family causing bacterial infection in the wound and the respiratory as well as the urinary tract [45].

4.2. Antifungal. The antifungal activity of the cajuput leaf essential oils had been determined against Fusarium oxysporum, Thanatephorus cucumeris, and Rhizopus oryzae [46]. The results showed that the oils were active against F. oxysporum with  $IC_{50}$  0.01–0.11 mg/mL and against Thanatephorus cucumeris with  $IC_{50}$  0.52–4.20 mg/ mL, but less active against R. oryzae as it only showed  $IC_{50}$  of 1.35–7.61 mg/mL. Fusarium oxysporum is a soilborne pathogen bacteria that cause vascular wilts in several plants [47]. Thanatephorus cucumeris which is classified as basidiomycetes also causes plant diseases to numerous agricultural and horticultural plants worldwide [48]. Rhizopus oryzae is a parasitic fungus that penetrates in the citrus fruit tissue through microwounds and bruises [49].

Along with the above studies, the antifungal assay of cajuput leaf oils was also determined against Fomitopsis palustris, Tinea versicolor, and Chaetomium globosum. According to Rini et al., the cajuput leaf oils demonstrated activities antifungal with  $IC_{50}$ 0.12-3.16 mg/mL, 0.01-0.06 mg/mL, and 0, 06-0.15 mg/mL for Fomitopsis palustris, Tinea versicolor, and Chaetomium globosum, respectively [50]. F. palustris enzymatically breaks down the cellulose of wood and leads to the plant disease namely brown rot [51], whereas Chaetomium globosum is an endophytic fungus assisting the cellulose decomposition of plant cells [52]. T. versicolor which is known as a turkey tail mushroom exhibits anticancer activities due to the polysaccharide constituents [53]. Candidiasis is a fungal infection which commonly occurs in female, particularly in the female reproductive organ. The cajuput leaf oils potentially eradicated the Candida albicans with MIC 62.5 µg/mL which is comparable to ketoconazole, the well-known synthetic drug used to treat candidiasis [54].

4.3. Antivirus. The potential antiviral activity of cajuput aqueous and methanol extracts were investigated against herpes simplex virus-1 (HSV-1) [55]. The aqueous extract demonstrated a 55.6% plaque reduction activity. Interestingly, at the same concentration ( $100 \mu g/mL$ ), the methanol extract exhibited a 100% plaque reduction defining the antiviral effect of such a plant against HSV-1. This potentially reduced the mouse skin lesions and the mortality of the

subject of the study for sure. A study by Farag et al. on HSV-1 was also conducted to evaluate the antiviral activity of the fresh leaves oil of cajuput. This supported the previous finding that cajuput was able to inhibit HSV-1 which infected African green monkey kidney cells (Vero cell) with 92% of inhibition [15].

4.4. Repellent Activity. Cajuput leaf oil demonstrated repellent activities against Aedes (43.2%), Anopheles (100%), and Culex mosquitoes (100%) during eight hours of mosquito exposure [56]. When the oil was tested into the larvae of Aedes, it showed the larvicidal activity up to 3.3% at a concentration of 50 ppm [57]. A similar study was conducted by Noosidum et al. confirming the repellent activity of the oil against female Aedes aegypti mosquitoes. A high escape rate of the mosquitoes was observed for up to 24 hours [58].

Further insect repellent activities of cajuput leaf oils against the vector of dengue and filariasis were reported [59, 60]. The oil was showing 97.5% repellency when it was exposed to *Lasioderma serricorne*, which was a serious pest of tobacco leaves cigarettes, cocoa beans, cereals, oilseeds, pulses, spices, and dried fruits [61]. Another study of the repellency effect of cajuput leaf oils against *Sitotroga cerealella* was also reported. The oil repelled up to 61% after the photo natural period of *Sitotroga cerealella*, a Lepidoptera insect which causes an unpleasant smell of grains [62]. Cajuput leaf oils were also reported to have repellent activities against *Plutella xylostella*, the most important pest in cabbage [63].

4.5. Wood Decomposing Organisms. Organic material decomposition is carried out by microbes or mesofauna. This process breaks down the materials into basic elements such as N, P, K, Mg, and Ca, which are important for soil nutrients [64]. The microbes or mesofauna are often utilized as wood decomposers especially for the lignin and cellulose matters [65]. These decomposers are divided into primary and secondary decomposers.

The primary decomposer is a mesofauna such as Collembola and Acarina which converts large particles into micronized sizes [66]. A wood decomposition process is also generally related to macrowood decomposers, which could be insects and termites [67]. When the trees are logged down, the decomposers start to attack the wood, especially to that sapwood which is rich in cellulose and carbohydrate. The moist environment further supports the existence of the wood decomposers in the trees [68]. Hollowish trees including rubber (*Hevea brasiliensis*), palm oil (*Elaeis sp.*), walnuts (*Canarium vulgare*), and fire tree (*Delonix regia*) are commonly attacked by ground/subterranean termites (genus of *Coptotermes*) [69].

The wood decomposition process quickly occurs once the trees died or fallen down. This is because neither defense nor a tree recovery is leading to the direct contact between the dead trees with the ground rich of decomposers. This attack is also performed by the genus of *Coptotermes* due to the chemical substance such as eugenol which attracts the termites to come over the trees [70]. Moreover, a derivative of eugenol, i.e., methyl eugenol ( $C_{12}H_{24}O_8$ ) has a function to stimulate the olfactory sensory organ in termites [71]. In conjunction with this, the termites have been successfully identified as a living wood attacker to cajuput which is the genus of *Odontotermes* [72]. Focusing on the cajuput tree, the *Macrotermes gilvus* termites attack is unavoidable even though the trees are still growing [73], especially to those of roots as well as the branches [74].

Muslich and Rulliaty examined the durability of the processed wood of cajuput from three types of decomposer's attack, i.e., dried ground termites (*Cryptotermes cynocephalus* Light), common ground termites (*Coptotermes curvignathus* Holmgren), and marine wood termites conclude that cajuput is resistant toward the attack of *Coptotermes curvignathus* but is vulnerable toward other decomposers [75]. This protective effect might be due to the chemical constituent such as cineole, melaleucin, and terpineol [76].

The secondary decomposers are mostly in the form of fungi, including Trichoderma reesei, Trichoderma harzianum, Trichoderma koningii, Phanerochaete chrysosporium, Cellulomonas, Pseudomonas, Thermospora, Aspergillus Niger, Aspergillus terreus, Penicillium, and Streptomyces [77]. According to Eriksson et al., fungus is the most active decomposer which immediately converts organic materials into simpler organic elements [78]. Suprapti and Djarwanto successfully identified some fungi attacked to cajuput including Pycnoporus sanguineus, Polyporus, Trametes, Schizophyllum commune, Chaetomium globosum, Marasmius, and Dacryopinax spathularia. Pycnoporus sanguineus is known to be the strongest attacker among them, followed by the genus of Polyporus [79]. Fortunately, although being attacked by fungi, the chemical composition especially the essential oils can block it. Sri et al. confirmed that the cellulose content of cajuput wood was still relatively high compared to other wood samples such as pine (Pinus merkusii), rubber (Hevea brasiliensis), and sengon (Paraserianthes falcataria) after inoculated by the fungus as the decomposer [80].

The wood decomposition is accelerated by the enzymatic process which is expressed by microorganism cells [81]. This enzymatic process includes hydrolysis as well as oxidation to degrade the cellulose [82] and to depolymerize the lignin [83]. This degradation and depolymerization reduce the particle size of cellulose and lignin while dissolving them into water. As in common enzyme, the catalytic process is limited by the substrate suitability, humidity, and temperature. On the one hand, a cellulose enzyme actively decomposes cellulose into its soluble form yielding cellodextrin (6C), cellobiose (4C), and glucose (2C) [84]. On the other hand, the activity of laccase and peroxidase degrade the lignin into a red color product containing quinone as an oxidized product of guaiacol [85]. The cellulose enzyme is

expressed by *Chaetomium* sp. [86], whereas the laccase and peroxidase enzyme are produced by the genus of *Trametes* [87].

4.6. In silico Prediction of the Antidecomposing Effect of *Cajuput*. In silico method can be potentially used to study the activity of chemical substances in cajuput oil regarding the inhibition effect to the decomposing microbial enzymes. Interaction between the 3D structure of the enzyme and the molecule structure of the chemical of interest can be well investigated by using computational methods [88, 89]. One of the methods is molecular docking, carried out by predicting the free energy of binding as well as its conformation between micromolecule (chemical) and macromolecule (enzyme) [90]. Once the chemical of the oil interacts with an enzyme (by giving a negative value in the binding energy along with its stable conformation), this conformation blocked the enzyme catalytic function in the decomposition process.

The 3D structure of the enzyme is collected from a protein data bank (http://www.rcsb.org), which is a form of cryopurified enzyme crystal [91, 92]. The crystal is then photographed in a three-dimensional form using an X-ray crystallography method. The docking software will be further used to predict the activity of such enzyme including their inhibition by using this 3D crystal. This technique is very popular in the drug discovery process through enzyme inhibition that blocks pathogenic diseases [93].

In this section, we predicted the capability of some major chemicals in cajuput to interact with cellobiohydrolase from Chaetomium thermophilum (PDB 4a05) [94], native laccase B from Trametes sp. (PDB 3kw7) [95], and lignin peroxidase from Trametes cervina (PDB 3q3u) [96]. The full methodology of this in silico prediction is provided in Supplementary Materials. Table 2 presents the docking result of 10 chemicals identified in cajuput against three enzymes expressed by fungi causing the wood decomposition process. The 10 chemicals have a diverse structure bearing cyclic monoterpene, sesquiterpene alcohol, allylbenzene alcohol, bicyclic sesquiterpene alcohol, acyclic monoterpene alcohol, and acyclic monoterpene which could diverse the binding energy as well as its binding conformation. Figure 5 illustrates the docking conformation of those 10 chemicals into the enzyme's pocket site.

According to the binding energy, all chemicals occupied the pocket site of the three enzymes; however, they would rather interact with cellobiohydrolase than the laccase and peroxidase due to the lower binding energy of cellobiohydrolase compared to laccase and peroxidase. This suggests that the hydrolysis reaction could be the main mechanism of chemicals inhibition to the enzyme, which further led to blocking the wood decomposition process. All chemicals also performed oxidation inhibition toward both oxidase

Chamicala		Binding energy (kcal/mol)	
Chemicals	Laccase	Peroxidase	Cellobiohydrolase
1, 8-Cineole	-5.6	-5.6	-6.4
α-Eudesmol	-6.3	-6.4	-7.8
β-Eudesmol	-6.5	-6.4	-7.8
Eugenol	-5.4	-6.2	-6.4
Guaiol	-6.9	-7.8	-8.5
Isoeugenol	-5.8	-6.5	-6.7
Linalool	-5.5	-5.6	-6
Lupene	-4.4	-9	-6.1
trans-β-Ocimene	-5.1	-5.8	-6
Viridiflorol	-6	-7	-8

TABLE 2: Binding energy of the interaction between chemicals in cajuput leaf oil and the enzymes (laccase, peroxidase, and cellobiohydrolase).



FIGURE 5: The overlaid positions of 10 chemicals into the pocket site of (a) cellobiohydrolase, (b) laccase, and (c) peroxidase. The proteins are presented as the surface form, and the ligands are presented as sticks from inside the proteins; yellow color represents carbon, red color represents oxygen, and white color represents hydrogen.

enzymes. Lupene, on the one hand, exhibited the lowest binding energy toward peroxidase among others. However, on the other hand, lupene demonstrated the highest binding energy toward laccase associating with its lowest interaction with the corresponding enzyme. Guaiol, which also is mostly deposited in conifers, showed the strongest affinity to either laccase or cellobiohydrolase. The 1, 8-cineole as the major component in cajuput leaves also showed negative values in the binding energy which is comparable to that of guaiol. This finding suggests that lupene, 1, 8 cineole, and guaiol play an important role in inhibiting the wood decomposing process.

The binding mode of guaiol into the pocket site of cellobiohydrolase is represented by hydrogen bond interactions between oxygen (OH)-Tyr170 and hydrogen (OH)-Asp176 (Figure 6(a)). However, the stable conformation on how guaiol performed the hydrogen bonds was supported by hydrophobic interaction between the sesquiterpene ring with Trp367. The interaction of guaiol with laccase (Figure 6(b)) was also elucidated showing H-bond interaction with Ile63. Nevertheless, the hydrophobic interaction was also performed through its sesquiterpene ring with Ile46, Phe93, Val48, and Phe97. In the interaction between lupene and peroxidase (Figure 6(c)), there was no H-bond interaction observed. However, the lowest binding energy is contributed by hydrophobic interaction with Leu233, Leu239, Val160, Leu172, Leu171, Val183, and Phe46.

#### 5. Discussion

Cajuput has been broadly used in healthcare management including pharmaceutical inhalation dosage form, topical liquid for body warming, and aromatherapy, mainly due to its bioactivity and aromatic flavors. The main part of cajuput is the leaves that produce essential oil containing various chemical substances such as 1, 8-cineole (eucalyptol), eugenol, isoeugenol, guaiol, linalool, and viridiflorol. The bioactivity of the chemicals varies due to their unique molecular structure such as chiral carbon, rigidity-flexibility, and stereoselectivity. The bioactivity of plants against microorganisms could be beneficial for many purposes, such as the development of medicine, product recycling process, and other waste management-based industry.

Cajuput leaves produce solid waste which is not efficiently reutilized unless for an organic fuel. Cajuput leaves appear not to be as biodegradable as other leaves. This may due to the residue of the essential oils or other chemicals which has antimicrobial activities against bacteria, fungi, and termites. The antimicrobial activities are potential for the waste product to be used as a wood-preserving agent.

#### International Journal of Forestry Research





FIGURE 6: The binding conformation of (a) guaiol-cellobiohydrolase, (b) guaiol-laccase, and (c) lupene-peroxidase. The proteins are presented as the line form, and the ligands are presented as thick stick forms; yellow color represents carbon, red color represents oxygen, and white color represents hydrogen.

Many experiments investigating the activity of cajuput essential oil as an antimicrobial agent have been performed. Moreover, the detailed molecular mechanism on how the plant fights against decomposition was well studied via in silico and in vitro experiments. Some major chemicals in cajuput essential oil were simulated using a computational molecular docking study, and as expected, several chemicals were performing strong affinities with the enzyme of the microorganism such as fungi. This molecular interaction can simply explain how the functional groups of the chemicals interact with the important amino acid that surrounded the pocket site of protein of interest [97]. Such interactions include hydrogen bond, hydrophobic bond, and even the strongest interaction, i.e., electrostatic interaction may exist. Every single interaction contributes to the free energy of binding. The lower the free energy of binding (larger negative value), the stronger affinity between chemicals and the protein would be. Furthermore, the stronger affinity of chemicals toward protein leads to a higher chance of cajuput to be the protein's inhibitor associates with its anti-biodecomposition effect.

The in silico study could also be applied in other plants having similar properties. Guaiol in conifers has antimicrobial [98] and insecticidal properties [99] due to the alcoholic properties in the structure. The strong interaction between OH of guaiol and Tyr170 as well as Asp176 of cellobiohydrolase has been well studied using in silico docking as confirmed in the in silico prediction section. Other rational on how the 10 ligands are in silico predicted to inhibit the activity of cellobiohydrolase are incorporated with in vitro results, that on the one hand, 1,8-cineole,  $\alpha$ -eudesmol,  $\beta$ -eudesmol, eugenol, and isoeugenol actively inhibit the mycotoxigenic fungus [100, 101]. On the other hand, linalool has been reported to have antifungal activity against *Candida albicans* [102].

#### 6. Conclusions

The solid waste product of cajuput leaves can be potentially managed as a wood-preserving agent due to the residue of essential oils which have antimicrobial and insecticidal activities. Further processing of the cajuput leaf solid waste may increase the economic value of it. Guaiol, lupene, and 1,8-cineole were predicted to be the chemicals having a responsibility to those biological properties as predicted by an in silico study. In a future study, in vitro experiments should be conducted to examine the corresponding enzyme activity in decomposing fungus against cajuput.

#### **Data Availability**

No data were used to support this study.

#### **Conflicts of Interest**

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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#### **Supplementary Materials**

S1: the procedure of molecular docking using AutoDock Vina Program. (*Supplementary Materials*)

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