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RESEARCH ARTICLE

The Coating of Mahogany Leaves Composite Using Clove Leaf Essential Oil as An Anti-Fungal *Ganoderma lucidum*

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ABSTRACT

This work has been carried out to use clove leaf oil as a composite coating component having anti-fungal properties. The mushroom growth testing was carried out with minimum inhibitory concentration (MIC) testing with variations in concentrations of 0%, 0.2%, 0.4%, 0.6%, 0.8%, up to 1% and obtained MIC values of clove oil found at a concentration of 0.4% in inhibiting the growth of white-rot fungi. Ganoderma lucidum mushroom is used as a weathering fungus applied in composites. The results of SSD testing showed that the growth of Ganoderma lucidum fungus began to be inhibited in composites coated with clove oil with a concentration of 0.6%. FTIR testing showed the presence of hydroxyl group (O-H), methylene group (C-H), alkenyl (C=C), and aromatic ring (C=C-C), GC-MS test results showed clove oil containing 67.40% eugenol, 17.42% beta-caryophyllene, 2.91% isopropenyl acetate, 1.56% caryophyllene oxide and 0.91% alpha-copaene. Based on research data, it shows that clove leaf oil can be used as an anti-fungal coating component in composites.

Keywords: Composite, Clove oil, Ganoderma lucidum, Antifungal.

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INTRODUCTION

In the last two decades, the use of wood has continued to increase along with population growth, the high demand for unbalanced timber with greening has a serious impact on forest destruction. This situation caused a crisis in the wood industry due to the scarcity of wood raw materials which made the price of wood raw materials very expensive and environmental problems that made the wood industry the main suspect against environmental damage[1], [2], so it is necessary to find other alternatives to wood [3]. Mahogany plant (Swietenia macrophylla King) is a tropical plant that is widely grown in Indonesia, mahogany plant belongs to the Meliaceae family. Each part of this plant has many uses and benefits for humans either as a medicine or other purpose [4]. Mahogany leaf waste in Indonesia is abundant, so far only in stockpiles and burned and a small part is used for compost [5].

Mahogany leaves are organic waste[5] that can be developed into composite materials because their quality is suitable for use as a substitute for wood materials used in particle board composites and the amount is abundant until many people just throw them away. Mahogany leaf waste can be used as a filler filled in certain binder polymers (matrices) to be processed into a strong and lightweight material through composite engineering [3]. The quality of composite particle boards can be improved by providing fiber reinforcement. Factors affecting the properties of composites include the type and shape of the particles, density, compressive strength, filler material, and binder used. Another factor affecting the quality of the composite is water absorption [3].

Essential oils are closely related to extensive biological activity, functioning as antioxidants, anti-inflammatory, antimicrobial, antifungal, antiviral, and antineoplastic [6]. Essential oil itself has many types such as clove leaf oil, clove steam oil, clove bud oil, cinnamon oil, vanillin, eugenol, caryophyllene and many more types of essential oils. Antifungal activity of clove oil, betel oil, eucalyptus oil, citronella oil, and peppermint has been shown to inhibit the activity of the fungi *Penicillium* sp., Aspergillus niger, and Aspergillus versicolor [7]. Several studies have been conducted on the use of clove oil, such as its antibacterial and antifungal properties in food packaging [8], improvement of fish feed efficiency [9], anesthetic for ornamental fish [10], and as antibacterial agent in toothpaste [11]. However, there are still few studies that utilize clove oil as a coating material for particleboard, such as clove oil as surface treatment for rubberwood particleboard [12], and clove oil as historic wood preservative [13]. The previous studies on the use of clove oil for wood surface treatment have almost never been used for composite boards made from leaf waste, which contain a high number of fungi.

Considering the potential and anti-fungal properties of clove oil, in this research, the surface of Mahogany leaf composites was coated with the clove oil. The goal is to make the composite of Mahogany leaf waste, which will be applied for various applications such as particleboard, have high resistance against fungi that potentially grow from within the composite [14].

METHODS

Materials

The material used in this study was mahogany leaf waste (Swietenia mahogany*)* obtained from Semarang State University. Polyurethane polymer as an adhesive material is obtained from Bratachem, Semarang. *Fiber* glass or glass fiber as a reinforcing material is obtained from Bratachem, Semarang, Indonesia. Clove leaf essential oil as an anti-fungal is obtained from PT Etherische. Ganoderma lucidum mushroom is obtained from the Indonesian Institute of Sciences (LIPI). All chemicals used in this study are purely analytical materials obtained from Merck (Germany).

Composite Synthesis

The method used is a simple mixing method, carried out in the Physics laboratory, Semarang State University. Mahogany leaves that have been ground until smooth as the main component are weighed as much as 15 grams, then mixed with fiber glass that has been cut into small pieces as much as 0.4 grams. The fiber glass used will function as a reinforcement in composite materials [15].

The polymer matrix component used for mixing is polyurethane consisting of polyols and diisocyanates of 1.25 grams. The three components are stirred until all the ingredients are mixed and no lumps occur. After mixing well, it is put into a container / mold that has been prepared beforehand, then pressed using a hydraulic press of 34.47 MPa.

Minimum Inhibitory Concentration Test

The minimum inhibitory concentration (MIC) test is a test performed to determine the concentration of oil that is able to inhibit the fungus from growing. This test was carried out at the Pharmaceutical Laboratory, Wachid Hasyim University and the Microbiology Laboratory, Semarang State University.

Preparing tools and materials that have been sterilized using an autoclave. Pour 10 ml of PDA (Potato Dextrose Agar) into a petri dish, then add each concentration of clove oil that has been prepared (0.2%, 0.4%, 0.6%, 0.8% and 1%) into a petri dish that has been filled with PDA using a micropipette of 1 ml. The petri dish is shaken simultaneously for 15 seconds until evenly distributed and allowed to solidify. Ganoderma lucidum mushrooms that have been bred for 5 days in a petri dish are taken slightly using loop needles or sterile tweezers and transfer them to a petri dish containing Potato Dextrose Agar (PDA) media and clove oil. Each treatment is made twice repetition. After all the concentration of oil has filled with mushrooms, next put a petri dish into the incubator and the observation is carried out until the seventh day.

The minimum inhibition of clove oil that can inhibit the growth of the fungus Ganoderma lucidum is calculated using the following equation:

$$\text{Inhibition (\%)} = \frac{CD.Control - CD\ Colony}{CD\ Control}\ x\ 100\%$$

where CD is the colony diameter (cm) [16].

Composite Coating

The clove oil used for composite coating is taken from the minimum concentration obtained through the MIC test. There are 6 variations of coatings that have been used, namely concentrations of 0%, 0.2%, 0.4%, 0.6%, 0.8%, and 1%. Coating on the composite is carried out by soaking the composite in clove oil to various concentrations of cloves for 30 seconds. The finished composite is coated, then dripped with fungal spores that have been bred on PDB (Potato Dextrose

Broth*)* media, then observed for 1 month using a Charge Coupled Device (CCD) microscope.

Sample Characterization and Clove Oil

Characterization of the functional groups of clove oil compounds using an FT-IR (Fourier Transform Infrared) spectrometer with the brand or type Perkin Elmer or Spotlight 400 Frontier at the Physics Laboratory of Semarang State University. Determining the content of compounds contained in clove oil using GC-MS (Gas Chromatography Mass Spectroscopy) with the Shimadzu QP 2010 SE at the Laboratory of the Islamic University of Indonesia, Yogyakarta. Determining the morphology and elements contained in the composite using SEM-EDX (Scanning Electron Microscopy-Energy Dispersive X-ray) type Phenom at the Physics Laboratory of Semarang State University.

RESULTS AND DISCUSSION

Composite Synthesis

The utilization of mahogany leaf waste is used as the basic material for making composites. Mixing between polyurethane polymer binders and fiber glass reinforcement is referred to as a simple mixing process. Mahogany leaf waste composite has tight pores and is finely textured, with diameter ± 5 cm as shown Figure 1 [17].

Figure 1. Composite Morphology

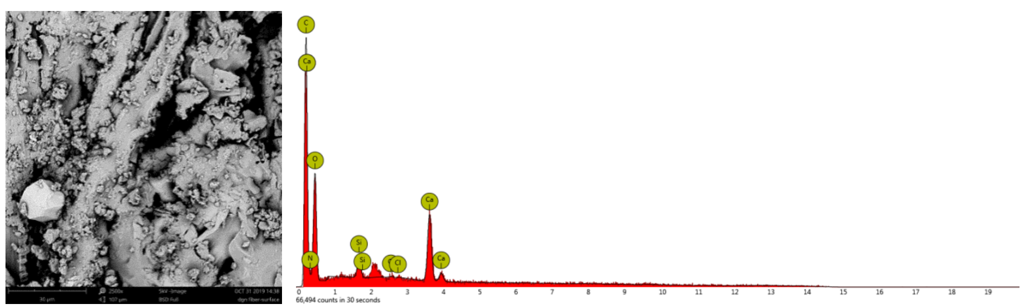

Figure 2. Composite SEM-EDX Test Results

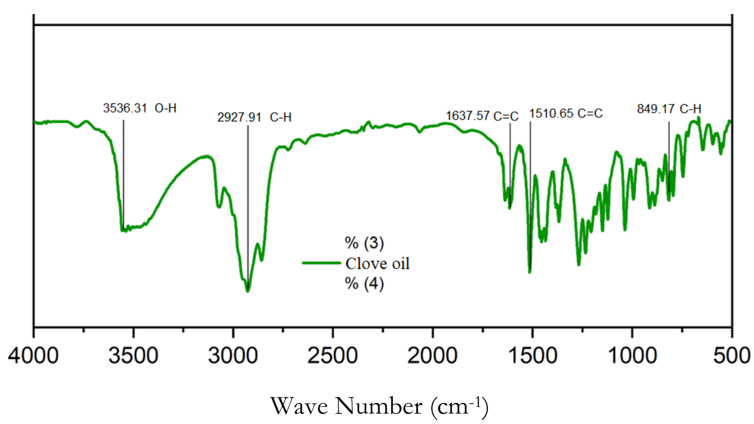

Figure 3. Clove oil IR spectrum

SEM-EDX Measurement

The morphology of the composite surface is shown Figure 2. The fiber glass contained in the composite has the shape of an elongated cylinder. Mahogany leaf waste powder and polyurethane are fused with fiber glass, proving during the simple mixing process that the materials are mixed homogeneously. The elements contained in the composite are carbon (C), calcium (Ca), oxygen (O), silicon (Si), nitrogen (N), chlorine (Cl), and aluminum (Al) [3], [17]. Carbon is the highest elemental content in the composite, namely 47.99%, calcium content of 34.17%, and oxygen content of 9.19%. The previous three elements are elements of mahogany leaf waste. Silicon has an elemental content of 4.75% which comes from fiber glass.

Clove Oil FT-IR Characterization

The FTIR spectrum in Figure 3 shows that cloves contain dominant elements, namely C, O, and H in the absorption band range 3536.31-843.02 cm^{-1}.

Clove oil absorption band widens in the area of 3650-3100 (cm^{-1}) with peak uptake in the area of 3536.31 cm^{-1} the area is a hydroxyl group region bound to the functional H-group caused by stretching O-H [18]. The hydroxyl group informs the presence of eugenol compounds contained in clove oil. The second sharp peak is formed in the spectrum region 2927.91 cm^{-1}.

Absorption in the asymmetric C-H range region indicates the presence of functional groups belonging to the alkyl group or alkenes [18]–[20]. Absorption at the second peak indicates the amount of caryophyllene contained in clove oil. In addition to the absorption of clove oil in the area of 3536.31 cm^{-1} and 2927.91 cm^{-1}, there was also an uptake in 1680-1620 (cm^{-1}) which shows the alkenyl group C=C. Subsequent uptake occurred in the area 1510-1450 (cm^{-1}), indicating the presence of aromatic compounds that are in the C=C-C group of the aromatic ring group (aryl), the aromatic compounds are indicated by the absorption band 1510.65 cm^{-1} [18].

In general the absorption band area indicated by the infrared spectrum has confirmed the presence of eugenol and caryophyllene in clove oil samples tested using the Fourier Transform Infrared (FTIR) test kit [21].

GC-MS Clove Oil Characterization Results

The results of the GC-MS analysis shown in Figure 4 show that there are several chemical compounds with high concentrations detected in clove oil.

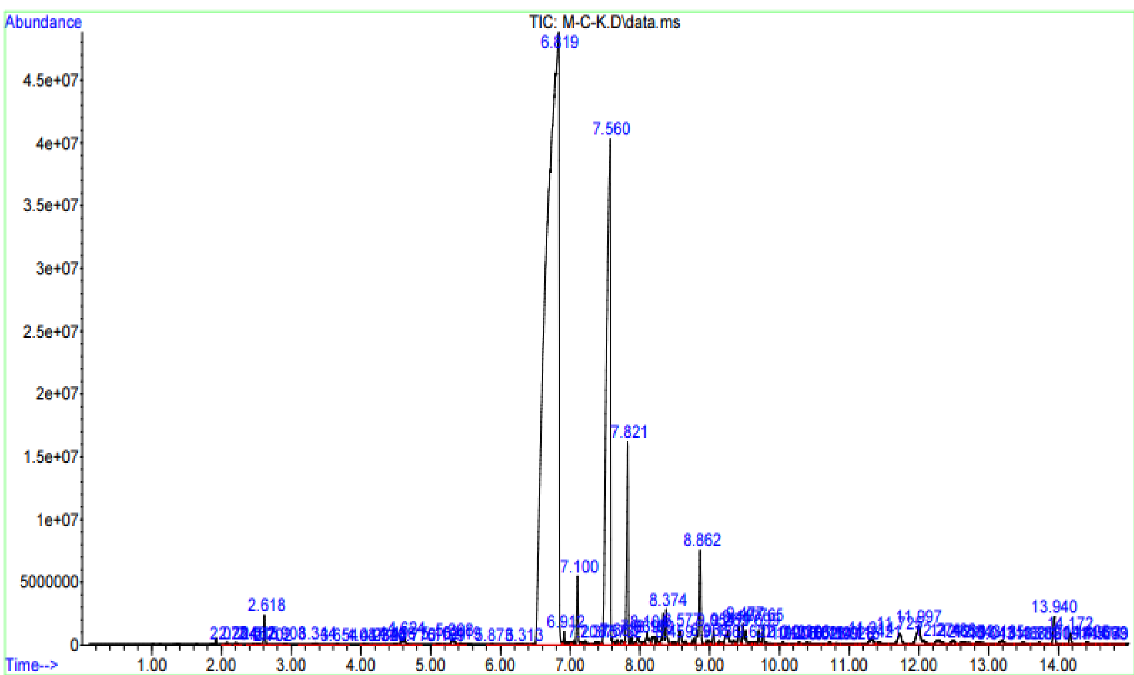

Figure 4. Clove oil GC-MS Chromatogram

The first peak was detected at a retention time of 6,819 which was identified as phenol, 2-methoxy-4-(2-propenyl) otherwise commonly known as eugenol. In clove oil eugenol has a very large percentage, which is 67.40%. Eugenol which has the molecular formula $C_{10}H_{12}O_2$ belongs to the group of organic compounds known as methoxyphenols [22], [23].

The second peak at a retention time of 7,560 minutes was identified as a beta-caryophyllene compound with the molecular formula $C_{15}H_{24}$. The compound beta-caryophyllene is a pale yellow liquid with an odor halfway between the smell of cloves and turpentine, commonly known simply as caryophyllene. Caryophyllene has a percentage of 17.42% and belongs to a class of organic compounds known as sesquiterpenoids [23].

The next compound detected in clove oil is isopropenyl acetate with the molecular formula C_5H_8O 2 detected at a retention time of 7.821 minutes having a percentage of 2.91%, then caryophyllene oxide. Caryophyllene oxide compounds with the molecular formula $C_{15}H_{24}O$ and molecular weight of 220.35 g/mol were detected at a retention time of 8.862 minutes with a compound percentage of 1.56%, and at a retention time of 7.100 minutes copaene compounds were identified with the molecular formula $C_{15}H_{24}O$ and found to be 0.91%. Clove oil testing using Gas Chromatography-Mass Spectroscopy (GC-MS), reinforcing the results of previous FTIR tests that clove oil contains eugenol and caryophyllene [23]-[26].

MIC Test and Composite Coatings

The results of the analysis based on Table 1 show that different concentrations affect the growth of fungi. Based on the percentage of inhibition, it is known that clove oil is able to inhibit fungi at a minimum concentration of clove oil of 0.4 %, in other words, the MIC value for Ganoderma lucidum mushrooms is 0.4%. White-rot fungi species can be 100% stunted in growth at oil concentrations above 1% for Ganoderma lucidum fungus . This shows that the more concentration of clove oil used, the greater its ability to inhibit the growth of fungal spores, especially white weathering fungi, namely the fungus Ganoderma lucidum [3], [17], [23].

This result is comparable to several previous studies, such as the clove oil utilization for dipping rubberwood and a concentration of 0.63% was found effective in protecting the wood against *Aspergillus* sp. and *Trichothecium* sp. fungi [12]. Another study also found that the use of clove oil for the preservation of historic wood was highly effective at a concentration of 5-10% [13].

Table 1. Percentage of clove oil inhibition to Ganoderma lucidum fungus on the 7th day.

Clove Oil Concentration (%)	Colony diameter (cm)	Inhibition (%)
0.0	1.50	0.00
0.2	1.50	0.00
0.4	0.85	43.33
0.6	0.70	53.33
0.8	0.40	73.33
1.0	0.00	100

The process of coating clove oil on the composite has been completed. The oil used is clove oil with a concentration that has been obtained from Minimum Inhibitory Concentration (MIC) testing. The growth of spores of the fungus Ganoderma lucidum that has been dripped on the composite surface, was observed microscopically using a CCD camera microscope with a magnification of 50x. Here are the results of the composite coating process using clove oil [3], [17], [23].

From observations made using a CCD microscope, it can be seen that there are samples with a large ` scale and some are shrinking. This is caused because the spores of the fungus Ganoderma lucidum injected on the composite surface are stunted by the growth process. In samples (a) and (b) with an oil concentration of 0% or a control concentration of 0.2%, the fungus Ganoderma lucidum can grow well and infect the composite [17].

As for samples (c), (d), and (e), where the composite has been coated with clove oil, Ganoderma lucidum mushrooms began to inhibit their growth process, especially in samples (d) and (e) with clove oil concentrations of 0.6% and 0.8%. Meanwhile, sample (f) of Ganoderma lucidum fungus has disappeared because it is unable to grow on composites with a clove oil concentration of 1%. This proves that clove oil can inhibit the growth of wood weathering fungi, especially white-rot fungi on composite materials instead of particle boards [17], [23].

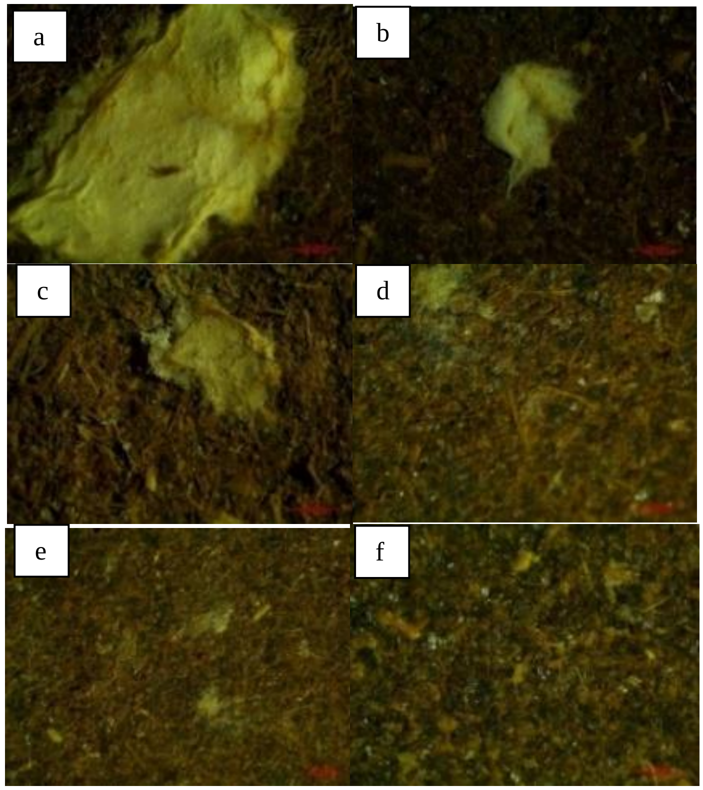

Figure 5. Composite observation results using a microscope with a magnification of 50x with oil concentration: (a) 0%; (b) 0.2%; (c) 0.4%; (d) 0.6%; (e) 0.8%; and (f) 1.0%

Mushroom Growth Rate Modeling

The following are the results of fungal growth modeling that have been obtained during MIC test.

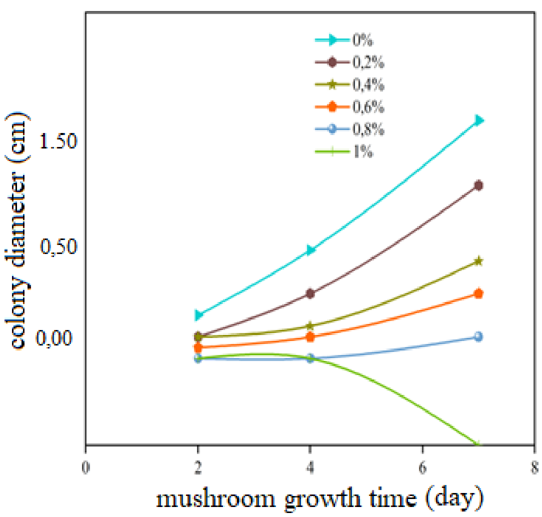

Figure 6. Modeling the growth rate of Ganoderma lucidum mushroom in various concentrations of clove oil.

From the results of graphic observations in Figure 6, it was found that the element of increasing the size of the mushroom d*S* is proportional to the size of the diameter of the mushroom colony *S*. In addition, the element of increasing the size of the fungus is also proportional to the time of its growth d*t*. The measurement value of the growth rate of the fungus shows an exponential decrease in the time at the time of inoculation. Mathematically the relationship can be written,

$$dS \propto S\, dt$$

or it can be written into

$$dS = \lambda S\, dt$$

with $\lambda =$ growth constant. Further, the calculations depicted by the graph in Figure 7 can be represented in Table 2.

Table 2. Results of fitting graphs of the growth rate of the fungus Ganoderma lucidum

Clove Leaf Oil Concentration (%)	S_0 (cm)	λ	R (%)
0.0	0.32	0.22	0.00
0.2	0.32	0.22	0.00
0.4	0.37	0.12	42.86
0.6	0.41	0.10	54.55
0.8	0.46	0.07	68.18
1.0	0.51	0.00	100

where S_0 is the diameter at the time of $t = 0$; and R is the ratio of fungal growth inhibition obtained by: $R = \frac{\Delta\lambda}{\lambda_0}$.

The ratio of inhibition of growing mushrooms indicates the ability of the oil to inhibit the fungus at a certain concentration. Table 2 shows that with a clove oil concentration of 0.4% it can inhibit mold by 42.86%. Mushrooms can be 100% stunted in growth at a concentration of 1% clove oil. The modeling fitting results using a differential equation of order 1 have a value of the ratio of inhibition of fungal growth that is close to the value of inhibition obtained during the MIC test.

CONCLUSION

Different concentrations of clove oil which affects the growth of the fungus Ganoderma lucidum on the composite. The more concentration of clove oil used, the greater the concentration of clove oil in inhibit the growth of fungi. Based on the research that has been done, the Minimum Inhibitory Concentration (MIC) value of clove oil is at a concentration of 0.4% with an inhibition of 43.33%. The MIC test also showed that the growth of the fungus began to be inhibited at a concentration of 0.6% clove oil. Meanwhile 100% inhibition was obtained at a clove oil concentration of 1.00% clove oil. This proves that clove oil can be used as component of composite or antifungal coatings. Modeling using a 1st order differential equation fungus growth inhibition ratio that is close to the inhibition value obtained during the MIC test, which is 42.86%. This suggests that a 1st order differential equation of fungi growth can be used to determine the predictive value of the inhibition of clove oil in inhibiting the growth of the fungus *Ganoderma lucidum*.

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