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A PRELIMINARY STUDY OF THE UTILIZATION OF CU(II) MODIFIED LIQUID SMOKE TO INHIBIT THE ACTIVITY OF WHITE-ROT FUNGI (*SCHIZOPHYLLUM COMMUNE FR*) IN A PINEWOOD IN-VITRO

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ABSTRACT: This study aims to examine the possibility of utilizing Cu(II) modified liquid smoke as an antifungal agent against *Schizophyllum commune Fr*. in a laboratory test. The liquid smoke was obtained through the pyrolysis of oil palm kernel shells at 300°C, 340°C, and 380°C. The analysis of liquid smoke as an antifungal agent was performed using potato dextrose agar (PDA) media and nystatin as positive controls. The white-rot fungi of *Schizophyllum commune Fr*. were isolated from fungally infected wood and regenerated for 5x24 hours. Observation of the inhibiting zone was done in 24 hours. The results showed that the presence of Cu(II) can increase the inhibiting zone of white rot fungus. The Cu(II)-modified liquid smoke was able to significantly inhibit the growth of white-rot fungi even in low liquid smoke concentrations. The highest inhibiting zone of *Schizophyllum commune Fr*. was obtained with 3% Cu(II)-modified liquid smoke (obtained from a pyrolysis temperature of 380°C).

Keywords: Liquid smoke, Wood damage, White-rot fungi, Inhibiting zone, Antifungal

1. INTRODUCTION

Liquid smoke is produced from the condensation of smoke resulting from the pyrolysis of wood containing acid, phenol, and carbonyl compounds [1], [2]. These compounds act as antibacterial [3], antifungal [4], [5], biopreservative [6], antioxidant [7], insecticide [8], and flavoring agents [9]-[11]. Compounds such as phenol and derivatives of carboxylate acid can produce differences in antifungal activities [12], [13]. Velmurugan et al. [14] report that their research shows that liquid smoke can inhibit the growth of *Ophiostoma polonicum, O. flexuosum, O. narcissi, and O. tetropii*. This means that liquid smoke has the potential to be used as an alternative to preserving wood because it can control microorganisms such as fungi and termites. The main compounds in liquid smoke are a phenolic and organic acid, which can work effectively to control microbial growth and increase the wood's resistance to fungi, such as white-rot (*Schizophyllum commune Fr*.). The white-rot fungus is capable of degrading lignins and wood components [15], making the wood brittle and significantly reducing the elasticity of its fibers. Moreover, this fungus grows quickly and easily, so it requires effective handling.

Current and commonly used wood preservatives are chromated copper arsenate (CCA) [16], copper azole (CuAz), and alkaline copper quaternary (ACQ) [17]-[19]. These compounds are made synthetically using several metal combinations. However, some of these substances have been discontinued because they are detrimental to the environment due to their dangerous metal contents, such as arsenic and chromium. Copper (Cu) has the best antimicrobial properties that do not harm the environment. A combination of organic biocides with antioxidants, such as metal, provide prospective alternatives for the development of wood-preserving materials [20]. This research is a preliminary study on the effect of Cu(II) modified liquid smoke. as an antifungal agent against *Schizophyllum commune Fr*. in-vitro, which is expected to benefit the wood preservation industry by discovering an environmentally friendly alternative to preserving wood.

2. METHODOLOGY

2.1 Processing the Liquid Smoke

The liquid smoke was produced based on previous research [6], [21]. Thus, 5 kg of oil palm kernel shells were processed in a pyrolysis reactor made of stainless steel with temperatures set between 300°C and 380°C. A schematic diagram of the pyrolysis process can be seen in Figure 1.

The resulting smoke was then condensed into a liquid called liquid smoke, which was then left for one day to separate from the tar content. The liquid smoke was then filtered through filtering paper to remove dirt and dust before it was analyzed using Gas Chromatography-Mass Spectrometry (GCMS-QP2010, Shimatsu, Japan).

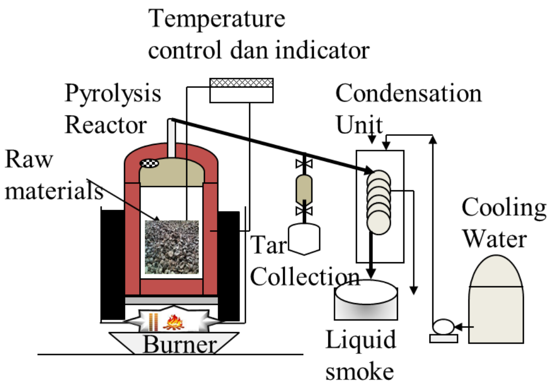


Fig. 1 Schematic diagram of the pyrolysis process [6].

2.2 Preparing the Cu(II)-Modified Liquid Smoke

The concentrations of liquid smoke used to test the antimicrobial effectiveness were 1%, 2%, and 3%. Each concentration was added into a $CuSO_4$ solution to create a Cu content of 0.2 M before it was homogenized. This Cu-modified liquid smoke was then characterized using a Fourier Transform InfraRed (FT-IR) test.

2.3 Processing the Potato Dextrose Agar (PDA) Solution

As much as 3.9 g of PDA powder was poured into an Erlenmeyer before 100 mL of distilled water was added. This solution was then heated on a hot plate with occasional stirring to mix the content well. The Erlenmeyer was then covered with cotton and paper and sterilized in an autoclave at 121ºC for 15 minutes [22].

2.4 Isolating the White-Rot Fungi

The fungi were extracted from the infected wood, cleaned and isolated in the PDA for 4-7 days, then inoculated into a new PDA for another 4-7 days before it was suspended in a Potato Dextrose Broth (PDB). Afterward, it was homogenized in a shaker for 4×24 hours. Finally, the fungi were placed under a microscope to identify their hypha.

2.5 Testing the Effectiveness of Liquid Smoke against *Schizophyllum Commune Fr.* Fungi

Sample testing was carried out using the diffusion disk method on paper disks with 6mm diameters. As much as 100 µl of the fungi's pure isolates were extracted and evenly dripped onto the PDA media using a triangle stir bar. Then, they were left for ±15 minutes. Afterward, each paper disk was dipped into 1%, 2%, and 3% Cu(II)-modified solutions and left for ±10 minutes to dry at room temperature. The discs were then lined up on the surface of the testing media using tweezers and incubated at room temperature for 24 hours. Afterward, the clear-colored area that formed on each disc was analyzed [23].

3. RESULTS AND DISCUSSION

3.1 Characteristics of Cu(II) in Liquid Smoke Using FT-IR

The composition of liquid smoke produced by pyrolysis at a temperature of-of 380°C is presented in Table 1. The liquid smoke mainly contains acetic acid, benzenamine, and phenolic compounds.

Table 1 GC-MS data for liquid smoke composition

No	R. Time	Area	%	Name
1	4,925	1175244109	25.74	Acetic acid (CAS) Ethylic acid
2	5,367	279748678	6.13	Hydrazine, 1,1-dimethyl- (CAS) N,N-Dimethylhydrazine
3	5,664	66706056	1.46	2-Propanone, 1-hydroxy- (CAS) Acetol
4	6,054	118809626	2.60	Acetic acid (CAS) Ethylic acid
5	7,122	373629213	8.18	Acetic acid (CAS) Ethylic acid
6	8,860	169255318	3.71	1H-Pyrazole, 3,5-dimethyl- (CAS) 3,5-Dimethylpyrazole
7	11,869	1416465628	31.02	Benzenamine (CAS) Aniline
8	12,633	155701085	3.41	2-Cyclopenten-1-one, 2-hydroxy-3-methyl- (CAS) Corylon
9	13,092	258903689	5.67	Phenol, 4-methoxy- (CAS) Hqmme
10	13,9	77275634	1.69	Phenol, 2,4-

Table 1 continued

	25			dimethyl- (CAS) 2,4-Xylenol
11	14,287	70803249	1.55	2-Methoxy-4-methylphenol
12	14,884	111127944	2.43	1,2-Benzenediol (CAS) Pyrocatechol
13	15,175	82991548	1.82	Phenol, 4-ethyl-2-methoxy- (CAS) p-Ethylguaiacol
14	15,925	138780247	3.04	Phenol, 2,6-dimethoxy- (CAS) 2,6-Dimethoxyphenol
15	17,801	71139682	1.56	1,6-Anhydro-Beta -D-Glucopyranose (Levoglucosan)

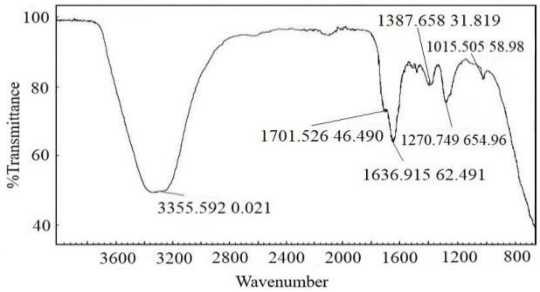
When the liquid smoke reacted with the 0.2 M of Cu(II) sulphate, it resulted in the color changes shown in Figure 2. There are two possible reactions between the copper and the organic compounds, since the liquid smoke that have the potential to become ligands, such as carbonyl derivatives of aniline, which are capable of forming complex compounds. First, these liquid smoke compounds play important roles in metal binding.

(a) before mixing (b) Cu-Liquid smoke

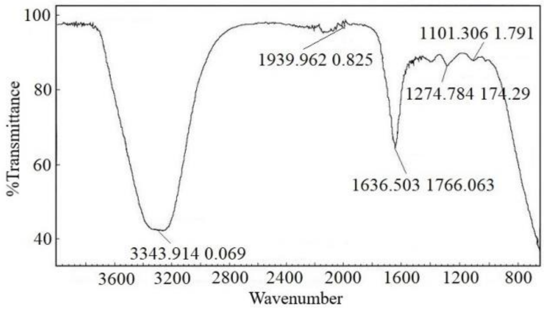
Fig. 2 The liquid smoke's changes in color.

Phenol can bind metal with its hydroxyl group, whereas carbonyl and acid can bind metal with their carbonyl and carboxylate groups. These organic compounds donate multiple free electron pairs (chelation). Second, copper is reduced from oxidation +2 to 0 due to the carboxylate's derivatives acting as redactors. This is shown in the color change seen when it returned to its basic, liquid smoke color after a while and from the presence of small particles that made the solution cloudy. These fine particles signify the formation of Cu(0) in very small amounts within the liquid smoke.

The FT-IR test results are shown in Figure 3. The bonding between the liquid smoke and the Cu (II) is indicated by the shifts and peaks recorded. In Figure 3(a), the peaks are recorded at 3355.9 cm^{-1} (signifying O-H (alcohol)), 1636.91 cm^{-1} (signifying C-C (aromatic)), 1387.66 cm^{-1} (signifying C-H (alkyl)), 1270 cm^{-1} (signifying C-O (phenol)), and 1015 cm^{-1} (signifying C-O). In Figure 3(b), the IR spectrum does not indicate meaningful changes in wave numbers, but the shifts in the peaks signify the binding of new compounds after adding Cu(II) sulphate into the liquid smoke. The shift from 1701 cm^{-1} to 1636 cm^{-1} and at 1274 cm^{-1} also signifies missing groups (-C-O). The new peak at 1989.96 cm^{-1} signifies reactions occurring. Wen et al. [26] state that the shifts indicate changes in functional groups.



(a)



(b)

Fig. 3 FT-IR results: (a) Liquid smoke, (b) Cu (II)-modified liquid smoke

3.2 Testing Liquid Smoke Against White-Rot Fungi

The diffusion disc method was used to test the Cu(II)-modified liquid smoke against the white-rot fungi. The effectiveness of the liquid smoke was shown by the formation of an inhibiting zone, which was a clean or clear area around the antifungal agent. The inhibiting zone was then compared with the diameters of the positive control, nystatin, and the negative control, distilled water.

The analysis showed that the modified liquid smoke's concentration level had a significant impacted on the resulting antifungal index: the higher the concentration, the higher the index was. This was seen from the formation of inhibiting zones on the discs in each concentration variation, wherein a stronger concentration produced a wider diameter. The liquid smoke that was formed from oil palm kernel shells had antibacterial and antifungal properties due to its alcohol, phenol, and organic acid compounds. Its antimicrobial properties were suspected to be able to disrupt the cytoplasm of fungal by destroying the membrane's surface pressure, causing a loss of active transport through the membrane and destabilizing various cell functions and structures.

The fungal growth inhibiting zones for the various concentrations of liquid smoke are shown in Tables 2-4. The copper-modified liquid smoke had significant effects on the diameters of the fungal growths. The inhibiting zones that formed using 1%, 2% and 3% of the copper-modified liquid smoke that was formed from pyrolysis at 300ºC had diameters of 21 mm, 22 mm, and 24 mm (on average), respectively.

Table 2 Inhibition zone testing of liquid smoke (temperature pyrolysis of 300ºC)

Treatment	Inhibition zone (mm)
Control (+)	23.3
Liquid smoke 1 % + $CuSO_4$	21
Liquid smoke 2 % + $CuSO_4$	22
Liquid smoke 3 % + $CuSO_4$	**24**
$CuSO_4$	15.3
Liquid smoke 2%	19.3
Control (-)	0

Table 3 Inhibition zone testing of liquid smoke (temperature pyrolysis of 340ºC)

Treatment	Inhibition zone (mm)
Control (+)	23.3
Liquid smoke 1 % + $CuSO_4$	22.3
Liquid smoke 2 % + $CuSO_4$	23.6
Liquid smoke 3 % + $CuSO_4$	**26**
$CuSO_4$	15.3
Liquid smoke 2%	19.3
Control (-)	0

Table 4 Inhibition zone testing of liquid smoke (temperature pyrolysis of 380ºC)

Treatment	Inhibition zone (mm)
Control (+)	23.3
Liquid smoke 1 % + $CuSO_4$	24.5
Liquid smoke 2 % + $CuSO_4$	26
Liquid smoke 3 % + $CuSO_4$	**27.3**
$CuSO_4$	15.3
Liquid smoke 2%	19.3
Control (-)	0

Pyrolysis at 380ºC resulted in inhibiting zone diameters of 24.3 mm, 26 mm, and 27.3 mm, respectively. The inhibiting zones formed using 2% liquid smoke and .02 M of $CuSO_4$ had diameters of 15.3 mm and 19.3 mm, respectively, while the positive and negative controls formed inhibiting zones of 23.3 mm and 0 mm in diameter, respectively.

The inhibiting zones formed using the same concentrations of copper-modified liquid smoke that were formed from pyrolysis at 340ºC had diameters of 22.3 mm, 23.6 mm, and 26 mm, respectively. The antifungal property tests conducted on the Cu-modified liquid smoke showed the highest inhibiting zones at a pyrolysis temperature of 380ºC. Oramahi et al. [27] stated that liquid smoke produced from oil palm trunks (pyrolyzed at 350°C) can inhibit the growth of the white-rot fungus, *T. versicolor*, at concentrations of 1.5%.

4. CONCLUSIONS

Liquid smoke formed from the pyrolysis of oil palm kernel shells at various temperatures has the potential to be used as an antifungal agent against *Schizophyllum commune Fr.*, as proven by the inhibiting zones that formed around the fungal growths. The copper-modified liquid smoke displayed significant effects in producing effective inhibiting zones, which are equally effective compared to the liquid smoke concentrations. This research shows that copper-modified liquid smoke can be categorized as a powerful and effective antifungal agent due to its ability to produce ≥20 mm inhibiting zones.

5. ACKNOWLEDGMENTS

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6. REFERENCES

[1] Zuraida, I. and Budijanto, S., Antibacterial Activity of Coconut Shell Liquid Smoke (CS-LS) and Its Application on Fish Ball Preservation. International Food Research Journal, Vol. 18, Issue 1, 2011, pp. 405-410.

[2] Faisal M., Chamzurni T., and Daimon H., A study on the Effectiveness of Liquid Smoke Produced from Palm Kernel Shells in Inhibiting Black Pod Disease in Cacao Fruit In Vitro. International Journal of GEOMATE, Vol. 14 Issue 43, 2018, pp. 36-41.

[3] Saloko, S., Darmadji, P., Setiaji, B. and Pranoto, Y., Antioxidative and Antimicrobial Activities of Liquid Smoke Nanocapsules Using Chitosan and Maltodextrin and Its Application on Tuna Fish Preservation. Food Bioscience, Vol.7, 2014, pp.71-79.

[4] Faisal M, Gani A, Husni, Baihaqi A, Daimon H., Pyrolysis of Oil Palm Kernel Shell into Liquid Smoke and Its Application to Control Anthracnose Disease on Chili (Capsicum annum L.). Journal of Engineering and Applied Sciences, Vol.11, Issue. 12, 2016, pp. 2583-2587.

[5] Ariestya, D.I., Swastawati, F. and Susanto, E., Antimicrobial Activity of Microencapsulation Liquid Smoke on Tilapia [Oreochromis Niloticus (Linnaeus, 1758)] Meat for Preservatives in Cold Storage (±5 C°). Aquatic Procedia, Vol.7, 2016, pp.19-27.

[6] Faisal M., and Gani A. The Effectiveness of Liquid Smoke Produced from Palm Kernel Shells Pyrolysis as a Natural Preservative in Fish Ball. International Journal of GEOMATE, Vol. 15 Issue 47, 2018, pp. 145-150.

[7] Faisal M., Gani A., Husni, and Daimon H., A Preliminary Study of the Utilization of Liquid Smoke from Palm Kernel Shells for Organic Mouthwash. International Journal of GEOMATE, Vol. 13 Issue 37, 2017, pp. 116-120.

[8] Gani, A., Husni, H., Baihaqi, A. and Faisal, M., Potential Development of Liquid Smoke from Oil Palm Solid Waste as Biofungicides. International Journal of Science and Engineering, Vol.7, Issue 1, 2014, pp.65-69.

[9] Tian, J., Zeng, X., Feng, Z., Miao, X., Peng, X. and Wang, Y., Zanthoxylum Molle Rehd. Essential Oil as a Potential Natural Preservative in Management of Aspergillus flavus. Industrial Crops and Products, Vol. 60, 2014, pp.151-159.

[10] Varlet V., Serot T., and Prost C., Smoke Flavoring Technology in Seafood. In Nollet LML, Toldra F. (Eds.), Handbook of seafood and seafood products analysis Boca Raton, FL: CRC Press. 2010, pp. 233-254.

[11] Guillen M.D., and Ibargoitia M.L., New Components with Potential Antioxidant and Organoleptic Properties, Detected for the First Time in Liquid Smoke Flavoring Preparations. Journal of Agricultural and Food Chemistry, Vol. 46, Issue 44, 1998, pp. 1276-1285

[12] Oramahi, H.A., Diba, F. and Yoshimura, T., Optimization of Production of Lignocellulosic Biomass Bio-Oil from Oil Palm Trunk. Procedia Environmental Sciences, Vol. 28, 2015, pp.769-777.

[13] Oramahi, H.A. and Yoshimura, T., Antifungal and Antitermitic Activities of Wood Vinegar from Vitex Pubescens Vahl. Journal of wood science, Vol. 59, Issue 4, 2013, pp.344-350.

[14] Velmurugan, N., Han, S.S. and Lee, Y.S., Antifungal Activity of Neutralized Wood Vinegar with Water Extracts of Pinus Densiflora and Quercus Serrata Saw Dust. International Journal of Environmental Research, Vol. 3, Issue 2, 2009, pp.167-176.

[15] Zhang, Z., Yang, T., Mi, N., Wang, Y., Li, G., Wang, L. and Xie, Y., Antifungal activity of monoterpenes against wood white-rot fungi. International Biodeterioration & Biodegradation, Vol. 106, 2016, pp.157-160.

[16] Ahn, S.H., Oh, S.C., Choi, I.G., Han, G.S., Jeong, H.S., Kim, K.W., Yoon, Y.H. and Yang, I., Environmentally Friendly Wood Preservatives Formulated with Enzymatic-Hydrolyzed Okara, Copper and/or Boron Salts. Journal of hazardous materials, Vol.178, Issue 1-3, 2010, pp.604-611.

[17] Gerengi, H., Tascioglu, C., Akcay, C. and Kurtay, M., Impact of Copper Chrome Boron (CCB) Wood Preservative on the Corrosion of St37 Steel. Industrial & Engineering Chemistry Research, Vol. 53, Issue 49, 2014, pp.19192-19198.

[18] Hingston, J.A., Collins, C.D., Murphy, R.J. and Lester, J.N., Leaching of Chromated Copper Arsenate Wood Preservatives: A Review. Environmental Pollution, Vol. 111, No. 1, 2001, pp.53-66.

[19] Freeman, M.H. and McIntyre, C.R., A Comprehensive Review of Copper-Based Wood Preservatives. Forest Products Journal, Vol 58, Issue 11, 2008, pp. 6-27.

[20] Schultz, T.P. and Nicholas, D.D., Development of Environmentally-Benign Wood Preservatives Based on the Combination of Organic Biocides with Antioxidants and Metal Chelators. Phytochemistry, Vol. 61, Issue 5, 2002, pp.555-560.

[21] Faisal M., Gani A., and AbuBakar, Removal

of Copper Ions from Aqueous Solution using Palm Shell Charcoal Activated by NaOH, International Journal of GEOMATE, Vol. 15 Issue 48, 2018, pp. 143-147.

[22] Berlian,Z., Aini, F., and Lestari, W.. Aktivitas Antifungi Ekstrak Daun Kemangi (Ocimum americanum L.) Terhadap Fungi Fusarium oxysporum Schlecht. (In Indonesia), Jurnal Biota, Vol. 2, Issue 1, 2016, pp.99-105.

[23] Sutton, S., Determination of Inoculum for Microbial Testing. Microbiology Topic, Vol. 15, Issue 3, 2011, pp. 49-53.

[24] Maga, J.A., The Flavor Chemistry of Wood Smoke. Food Reviews International, Vol.3, Issue 1-2, 1987,mpp.139-183.

[25] Girard, J.P., Technology of Meat and Meat Product Smoking, Ellis Harwood, New York, 1992, pp. 162-201.

[26] Wen, Y., Huang, W., Wang, B., Fan, J., Gao, Z., and Yin, L. Synthesis of Cu Nanoparticles for Large-scale Preparation. Materials Science and Engineering: B, Vol. 177, Issue 8, 2012, pp. 619-624.

[27] Oramahi, H.A., Yoshimura, T., Diba, F. and Setyawati, D., Antifungal and Antitermitic Activities of Wood Vinegar from Oil Palm Trunk. Journal of Wood Science, Vol. 64, Issue 3, 2018, pp.311-317.