FORESTRY IDEAS, 2022, vol. 28, No 2 (64): 370–379

Fungal inhibition of *Pinus merkusii* cone extracts against *Phanerochaete chrysosporium*

Masendra[1*], Agus Ngadianto[1], Ridla Arifriana[1], Fanniyatul Maulidia[1], Wakhdani Minorita[1], Brandon Aristo Verick Purba[2,3], and Ganis Lukmandaru[2]

[1]Department of Bioresources and Veterinary Technology, Vocational College, Universitas Gadjah Mada, Jl. Yacaranda, Yogyakarta 55281, Indonesia. [*]E-mail: masendra@ugm.ac.id
[2]Department of Forest Products Technology, Faculty of Forestry, Universitas Gadjah Mada, Jl. Agro No.1, Bulaksumur, Yogyakarta 55281, Indonesia.
[3]Natural Resources Conservation Center of West Kalimantan (BKSDA Kalimantan Barat), Jl. Ayani 121, Pontianak 78124, Indonesia.

Received: 05 April 2022 / Accepted: 27 October 2022 / Available online: 07 November 2022

Abstract

This study aims to identify the extractives from *Pinus merkusii* Jungh. & de Vriese cone together with their evaluation for antifungal agent. The pine cone was obtained from Temanggung, central Java, Indonesia. The pine cone was grinded to size of 40 mesh and was extracted with *n*-hexane, ethyl acetate, and methanol in a Soxhlet apparatus. The antifungal test was performed through growth inhibition of cone extracts against *Phanerochaete chrysosporium* Burds. (white-rot) and the extractive responsible for antifungal was elucidated by GC-MS. The antifungal activity measurement showed that *n*-hexane extract as the highest inhibition with IC_{50} of 637.74 μg·ml^{-1}. The analysis of *n*-hexane extract through GC-MS exhibited diterpenoid as dominant compound and they were characterized as pimaric acid and abietic acid TMS. Therefore, the presence of terpenoids in *P. merkusii* cone extracts are potential for natural preservative.

Key words: lipophilic, natural preservative, pine, resin acids, terpenoids, white-rot.

Introduction

White-rot (*Phanerochaete chrysosporium* Burds.) is one of the organisms that caused detrimental effect on wood industry. This type of fungal disease caused depolymerisation of hemicellulose and lignin components, leaving undegraded crystalline cellulose hence its white colouration at the advance stages of decay (Goodell et al. 2020). The utilisation of wood as material for furniture and other uses proposed the application of wood preservative to protect from the organisms. In another hand, the uses of synthetic preservative triggers side effect on human and environment. Therefore, the alternative way includes researching natural preservatives from plants. The potential of natural preservatives from plants has been reported from previous studies; phenolics (Zabka and Pavela 2013, Pizzolitto et al. 2015) and lipophilic such as essential oils (Srivastava et al. 2008) as antifungal against genus

Aspergillus. In the case of white-rot, plant extracts also were applicated as preservative agent (Da Silveira et al. 2017, Xie et al. 2017). Due to the white-rot attacks affects in decreasing the mechanical, physical, and chemical properties of wood, the use of white-rot such as *Ph. chrysosporium* for model laboratory test also has been recorded (Oliveira et al. 2010).

Pine cone is a reproductive organ produced by *Pinus merkusii* Jungh. & de Vriese and other species from Pinophyta division. It is composed by lignin, cellu lose, resins, and other organic compounds. The woody female cone is a common biomass waste in pine plantations. In previous works, pine cone was reported to contain hydrophilics and lipophilics extractives such as tannins, flavonoids, monoterpenes, as well as diterpenes (Kilic et al. 2011, Xu et al. 2012, Zhao et al. 2019). Several bioactivities such as antibacterial, antitumor, immunoregulatory, and antifungal have been reported in pine cone from various species (Ulukanli et al. 2014, Yi et al. 2017).

In Indonesia, especially in Java Island, pine forest (*Pinus merkusii*) sited of 476,126 ha and thus the material of pine cone presence is in huge amounts (Masendra et al. 2018). However, information on the fungal inhibition effect of *P. merkusii* cone extract is still limited. Therefore, the purpose of this study is to identify *P. merkusii* cone extractives responsible to inhibit the growth of white-rot for future development as natural preservative against fungi.

Material and Methods

Cone collection and extraction

Air dried of *P. merkusii* cone (tree age: 30-year-old) was collected from Temanggung, Central Java, Indonesia (7°14' S, 110°2' E). The site elevation is 500 m a.s.l., precipitation of 3000–3500 mm and annual temperature of 24–30 °C. The pine cones were sieved in size of 40 mesh and 53.5 g of dry sample was successively extracted with *n*-hexane, ethyl acetate, and methanol for 6 h using Soxhlet equipment. To obtain dried extract the solution sample was evaporated by evaporator machine and then was weighed as extractive content (percentage of initial sample).

Fungal inhibition test

Ph. chrysosporium was chosen for antifungal activity. Its strain IPBCC 930259 was collected and purchased from Institute Pertanian Bogor Centre Collection, West Java, Indonesia. The fungus was pre-cultured on PDA (potato dextrose agar; Aldrich, Germany) in Petri dish of 90 mm diameter for 7 days at 25 °C. In this experiment the medium was autoclaved at 120 °C for 20 min. The performance of antifungal extracts against *Ph. chrysosporium* referred to method described by Lukmandaru (2013). 300 µL extract sample was spread on the surface of 20 mL PDA medium in Petri dish. After the solvent drying, *Ph. chrysosporium* from pre-cultured PDA was inoculated. The fungus was incubated at 25 °C and 70 % relative humidity for 5–7 days. The blank sample was conducted in the same method with replacing the extract by solvent only. The incubation was stopped when the growth of fungus in blank sample was 100 %. In this experiment, a standard of biocide/fungicide (Bioindustries, Yogyakarta) was compared for control positive. It consists of methylene bis thiocyanate and 2-thyocyanomethyl thiobenzothiazole. The growth inhibition (*GI*) of sample was cal-

culated through equation (1) as follows:

$$GI = \frac{A_1}{A_0} \cdot 100,\ \% \qquad (1)$$

where: $A_1 = \frac{\pi \cdot d_1^2}{4}$ is area of sample in cm^2 and d_1 is diameter of sample growth in cm; $A_0 = \frac{\pi \cdot d_0^2}{4}$ is area of blank in cm^2 and d_0 is diameter of blank growth in cm.

The fungal inhibition was performed in three replications and three concentrations to calculate IC_{50} (the concentration that inhibit 50% activity). The concentration of 500, 1000, and 2000 $\mu g \cdot mL^{-1}$ were used to performed IC_{50}. In addition, the performance of IC_{50} calibration from *n*-hexane, ethyl acetate, and methanol extract, were $y = 0.037x + 25.8$, $y = 0.0191x - 5$, and $y = 0.0449x + 12$, respectively. All equations have $R^2 > 0.9$, where x is IC_{50} in $\mu g \cdot mL^{-1}$ and y is 50 $\mu g \cdot mL^{-1}$.

Gas chromatography mass spectra (GC-MS study)

The highest value of *Ph. chrysosporium* inhibition from the pine cone extracts was analysed with GC-MS. The *n*-hexane extract was injected for identification of extractive. It was silylated according to method described by Preciado et al. (2016). Briefly, 1 mg *n*-hexane extract was dissolved in 50 µL pyridine. Into solution sample, 100 µL solution consisted of N,O-bis (trimethylsilyl) acetamide 99 µL and trimethylchlorosilane 1 µL were added. The sample was put in oven with 100 °C for 30 min. After cooling process, 1 mL *n*-hexane was added to the sample solution. For analysis, 1 µL sample was injected to the GC-MS machine using a GCMS-QP 2010 (Shimadzu, Japan). Gas chromatography condition was Rtx-5MS capillary column (30 m × 0.25 mm I.D. and 0.25 µm; GL Sciences, Tokyo, Japan). The sample detection temperature was 280 °C and column temperature was set from 170 °C (1 min) to 280 °C at 5 $°C \cdot min^{-1}$. Injection temperature of 200 °C was set and acquisition mass range was made 50–500 atomic mass units with helium as gas carrier. In addition, the split ratio of GC-MS machine was made of 20. MS of *n*-hexane extractives was compared with NIST11 (2011) library and literature (Azemard et al. 2016, Masendra et al. 2018). Furthermore, the calculation of each compound was done using peak relative method (Masendra et al. 2021b).

Statistical analysis

One-way ANOVA was used to observe the significancy value of solvent. ANOVA was performed using SPSS software (IBM, USA) with 95% confidence level. Tukey-HSD was applicated to analyse the data with significant difference.

Results and Discussion

Extractive content

Figure 1 shows cone of *P. merkusii* abundance in hydrophilic extracts and lipophilic extract. The methanol extractive gave the highest value of 6.35 %, while ethyl acetate gave the lowest percentage of 1.95 %. The lipophilic extraction or *n*-hexane extraction yielded 3.11 % extract. The extractive content of *P. merkusii* cone was reported in a previous work (Masendra et al. 2021a). In comparison, the methanol extractive of *P. merkusii* cone was higher than acetone, toluene-ethanol, and water of wood (Wijayanto et al. 2015). Further, *n*-hexane extractive content of *P. merksii* cone was higher than *n*-hexane extract of bark (Masendra et al. 2018), but was low-

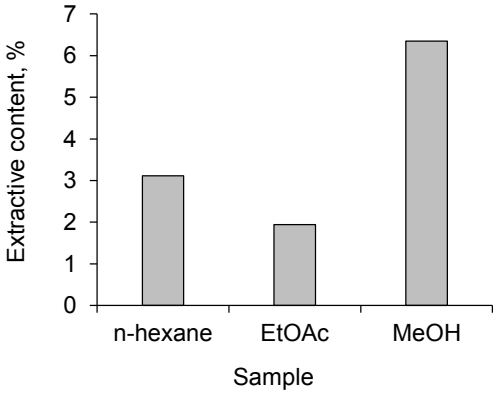

Fig. 1. Yield extractive of *P. merkusii* cone.
Note: EtOAc is ethyl acetate, MeOH is methanol (Masendra et al. 2021b).

er than *P. merkusii* wood dichloromethane extract (Wijayanto et al. 2015).

Antifungal assay and elucidation of extractive compounds

IC_{50} of pine cone extracts is displayed in Figure 2. The *n*-hexane extract exhibited highest inhibition 637.74 μg·mL^{-1}, followed by methanol (851.22 μg·mL^{-1}) and ethyl acetate (3446.01 μg·mL^{-1}). Further comparison with positive control of biocide (IC_{50} of 260 μg·mL^{-1}), the *n*-hexane extract was two times lower. The higher value of inhibition in *n*-hexane and methanol extracts than ethyl acetate extract indicates the lipophilic and hydrophilic compound involve in stopping the white-rot growth.

Due to higher inhibition of fungal in *n*-hexane extractive, thus, this lipophilic extractive was further discussed. The detection of *n*-hexane extract constituent found out four compounds (Fig. 3). The compound 1 was detected as sesquiterpene and compound 2–4 were identified as diterpenes through NIST11 library comparison (Table 1).

In Table 1 the compound with higher proportion in *n*-hexane extract was diterpene rather than sesquiterpene. Therefore, the characterisation of diterpene (compound 2–4) was further elucidated. The compound 2 showed fragment of mass-to-charge ratio (m/z) 374, 359, 309, 257, 241, 121, 73. The compound 3 proposed fragment of m/z 374, 359, 256, 241, 213, 185, 121, 73, and the last compound (4) had fragment of m/z 372, 359, 257, 239, 141, 105, 91, 73. The other fragmentations of compound 2–4 are displayed in Figure 4, while their comparation of mass spectra with previous works (Azemard et al. 2016, Masendra et al. 2018) is shown in Table 2.

Mass spectra interpretation of compound 2–4 were further discussed in this section. The appearance of m/z 73 in compound 2–4 suggests these compounds have TMS (-C_3H_9Si) in their structures (Fig. 5 for

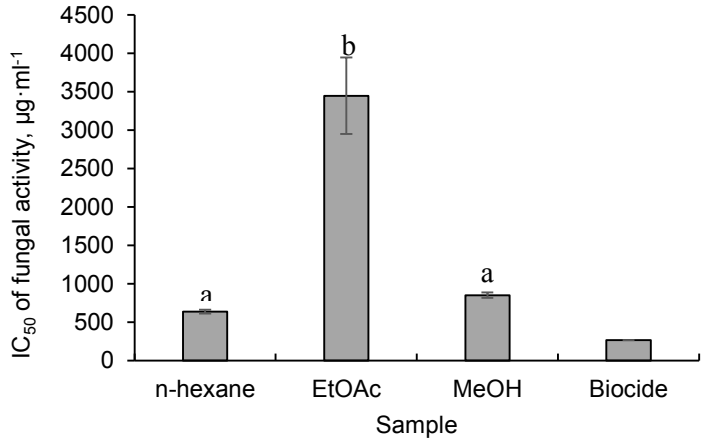

Fig. 2. Fungal inhibition from *P. merkusii* cone extracts.
Note: Different letters are statistically different at $p < 0.05$.

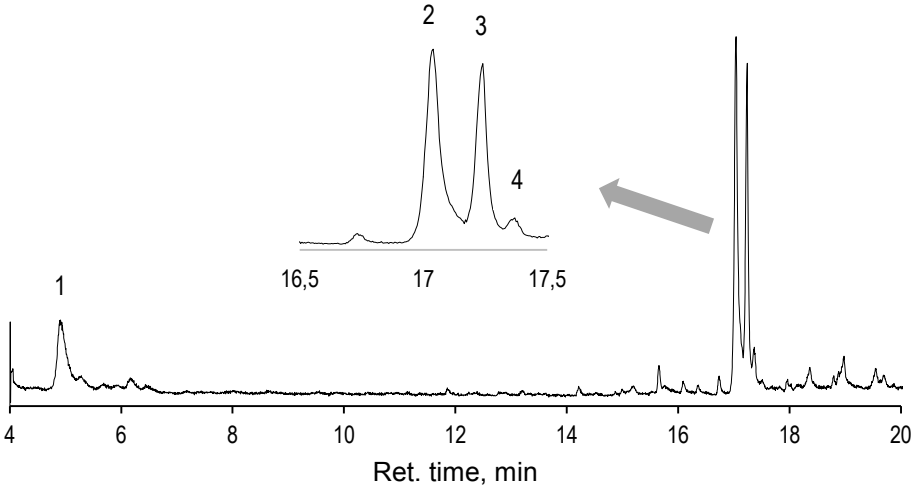

Fig. 3. GC-MS chromatogram of *P. merkusii* cone.
Note: compound 1 sesquiterpene, compound 2–4 diterpenes.

Table 1. Cone extract of *P. merkusii* constituents.

Compound	Constituents	Concentration, %	Group	Similarity index, %
1	Trans-longipinocarveol	12.91	Sesquiterpene	80
2	Pimaric acid TMS	49.12	Diterpene	80
3	Abietic acid TMS	34.43	Diterpene	80
4	Dehydroabietic acid TMS	3.54	Diterpene	88

Note: TMS is trimethylsilyl.

chemical structure of terpenoid detected in *P. merkusii* cone extract). Compound 2–4 showed total molecular peak (M^+) at m/z 374 (compound 2 and 3) and m/z 372 (compound 4). The presence mass spectra of m/z 359 [M^+ - m/z 15] in compound 2 and 3 indicated the loss of m/z 15 molecules and thus the compound 2 and 3 contain methyl (-CH_3) in their structure. While in compound 4, the m/z 372 to m/z 359 is indicated to the loss of m/z 13 molecules or methine (=CH-) in its structure. The next mass spectrum that appears in compound 2–4 is m/z 257 ($C_{19}H_{29}$) and m/z 256 ($C_{19}H_{28}$). Both m/z 257 and m/z 256 appear due to the loss of m/z 117 or 115 molecules ($C_4H_9O_2Si$) from m/z 374 or m/z 372. Moreover, the spectra in compound 2 and 3 have m/z 241 that estimated from the reduction of m/z 256 by m/z 15, while m/z 239 is made from the reduction of m/z 257 by m/z 18. The m/z 15 and m/z 18 is associated to the loss of methyl in compound 2 and 3, and water in compound 4. The other detected fragment in compound 2 and 3 is m/z 213. This fragment is made from reduction of m/z 241 by m/z 28, while m/z 211 in compound 4 is made from reduction of m/z 239 by m/z 28. The m/z 28 is associated to the loss

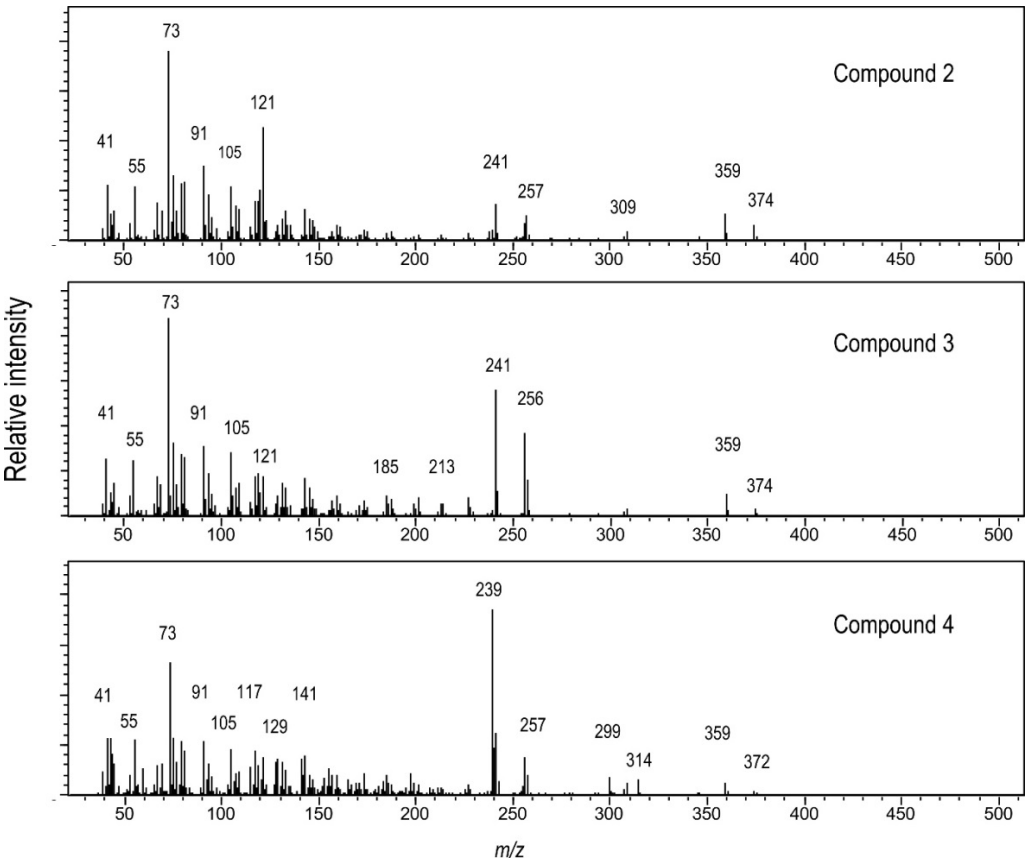

Fig. 4. Mass spectrum of compound 2–4.

Table. 2. Mass spectra comparison of compound 2–4 with literature.

Constituent	Mass spectra fragmentations
Pimaric acid TMS[a]	374 $[M]^+$ (10), 359 (27), 309 (3), 257 (19), 241 (19), 121 (100), 105 (14), 91 (15.1), 73 (95), 55 (22), 41 (5)
Compound 2	374 $[M]^+$ (8), 359 (13.7), 309 (3.3), 257 (13.8), 241 (16.9), 121 (55.5), 105 (22.3), 91 (33.3), 73 (100), 55 (24.2), 41 (21.1)
Abietic acid TMS[b]	374 $[M]^+$ (9), 359 (8), 256 (100), 241 (86), 213 (35), 185 (64), 157 (9), 143 (15), 129 (9), 105 (9), 91 (9), 73 (5)
Compound 3	374 $[M]^+$ (2.9), 359 (9.6), 256 (47.6), 241 (69.3), 213 (6.3), 185 (10.5), 157 (8.6), 143 (18.4), 129 (9.8), 105 (29.3), 91 (32), 73 (100)
Dehydroabietic acid TMS[a]	372 $[M]^+$ (15), 359 (11), 314 (2), 299 (2), 257 (11), 239 (100), 141 (10), 105 (3), 91 (4), 73 (27), 55 (5)
Compound 4	372 $[M]^+$ (2), 359 (6), 314 (7.5), 299 (9.6), 257 (11), 239 (100), 141 (15), 105 (20), 91 (25), 73 (65), 55 (25.3)

Note: [a] – Masendra et al. (2018), [b] – Azemard et al. (2016).

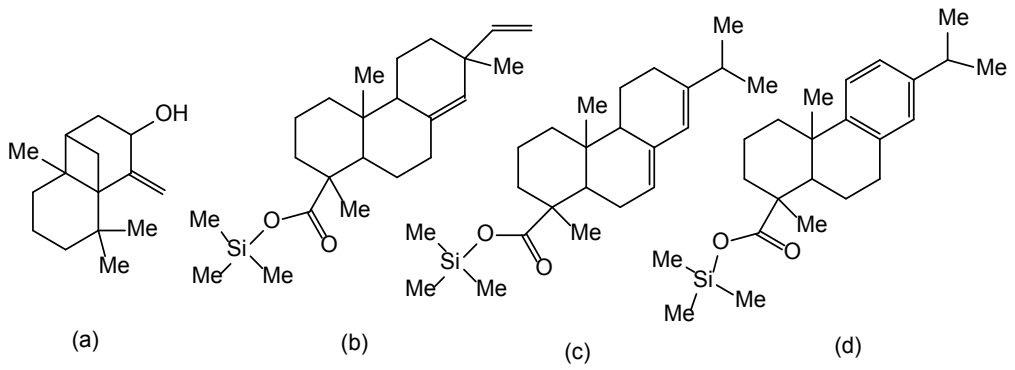

Fig. 5. Chemical structure of sesquiterpene and diterpenes.

Note: longipinocarveol (a), pimaric acid (b), abietic acid (c), and dehydroabietic acid (d) in TMS derivative.

of C_2H_4 (ethene). Hence, m/z 374 is predicted to be $C_{23}H_{38}O_2Si$ while m/z 372 is $C_{23}H_{36}O_2Si$. Therefore, these compounds were categorised as diterpenic compounds that contain four isoprenes (C_5H_8). Furthermore, the fragments of compound 2–4 were compared to previous work (Azemard et al. 2016, Masendra et al. 2018) and they were identified as diterpenic TMS derivative compounds, namely, pimaric acid for compound 2, abietic acid for compound 3 and dehydroabietic acid for compound 4 (Table 2). In addition, the comparison compound 2–4 with another genus *Pinus* was made. It was shown that the terpenic compound dominant in the *P. merkusii* cone is pimaric acid and abietic acid. The presence of those diterpenoids also was detected in the cone of *P. armandii* (Yang et al. 2010), *P. halepensis*, *P. pinea*, *P. sylvestris*, *P. nigra*, and *P. brutia* (Kilic et al. 2011).

The detection of the extractive in *P. merkusii* cone such as lipophilic and hydrophilic compound could be responsible as plant protector against microorganisms. In addition, in correlation with constituent of *n*-hexane and methanol extracts, the high fungal inhibition may due to the presence of diterpenoids and phenolics. The terpenoid especially diterpenic acids from *P. merkusii* cone such as pimaric acid TMS and abietic acid TMS were amounted in high concentration. Previously, it was reported that terpenic compounds from essential oil inhibited white-rot fungus (Xie et al. 2017). Related to the hydrophilic compounds, a previous work (Masendra et al. 2021a) reported that methanol extract of *P. merkusii* cone contained total phenols (54.5 %) and total flavonoid (3.4 %) as well as the detection of phloroglucinol and catechin. The phenolics in the *P. merkusii* cone might also act as fungal inhibitor (*Ph. chrysosporium*).

The mechanism of extractives in inhibiting of fungus growth could be defined by their lipophilicity and hydrophilicity. A previous work reported that lipophilic compound of eugenol, thymol, isoeugenol, and hydrophilic compound of vanillin act as inhibitor for *Fusarium verticillioides* (Sacc.) Nirenberg mycelia (Dambolena et al. 2012). The appearance of the mentioned properties is known by the presence of hydroxyl (-OH) for hydrophilicity and methyl for hydrophobicity in the compounds. In the phenolics and terpenes, it

was described that the presence of -OH and methyl group effected the fungal and bacterial activity (Sekine et al. 2009, Zengin and Baysal 2014, Pizzolitto et al. 2015, Masendra et al. 2021c). Therefore, in the current study, the presence of methyl group in the compound 2–4 as well as the presence of -OH group in trans-longipinocarveol, phloroglucinol, and catechin attribute in inhibiting the growth of mycelia from *Ph. crysosporium*. The presence of those compounds in *n*-hexane and methanol extracts suggests the pine cone of *P. merkusii* is potent for natural preservatives compound.

Conclusions

The fungal test of *P. merkusii* cone extracts against *Ph. chrysosporium* exhibited inhibition activity especially in *n*-hexane and methanol extracts. The investigation of *n*-hexane extractive detected terpenic compounds in the cone of *P. merkusii* and they were identified as trans-longipinocarveol, pimaric acid, abietic acid, and dehydroabietic acid. The higher presence of pimaric acid and abietic acid in *P. merkusii* cone indicated their role were dominant in inhibiting the growth of *Ph. crysosporium*. The presence of methyl group in both pimaric and abietic acid together with their lipophilicity suggested to have responsibility in stopping the fungus growth. The research concludes that *P. merkusii* cone is potential for future natural preservative development against white-rot, especially to *Ph. crysosporium*.

References

Azemard C., Menager M., Vieillescazes C. 2016. Analysis of diterpenic compounds by GC-MS/MS, contribution to the identification of main conifer resins. Analytical and Bioanalytical Chemistry 408(24): 6599–6612. doi: 10.1007/s00216-016-9772-9

Da Silveira A.G., Santini E.J., Kulczynski S.M., Trevisan R., Wastowski A.D., Gatto D.A. 2017. Tannic extract potential as natural wood preservative of *Acacia mearnsii*. Annals of the Brazilian Academy of Sciences 89(4): 3031–3038. doi: 10.1590/0001-3765201720170485

Dambolena J.S., Lopez A.G., Meriles J.M., Rubinstein H.R., Zygadlo J.A. 2012. Inhibitory effect of 10 natural phenolic compounds on *Fusarium verticillioides*. A structure-property activity relationship study. Food Control 28(1): 163–170. doi: 10.1016/j.foodcont.2012.05.008

Goodell B., Winandy J.E., Morrell J.J. 2020. Fungal degradation of wood, emerging data, new insights and changing perceptions. Coatings 10(12): 1211–1219. doi: 10.3390/coatings10121210

Kilic A., Hafizoglu H., Donmez I.E., Tumen I., Sivrikaya H., Reunanen M., Hemming J. 2011. Extractives in the cones of *Pinus* species. European Journal of Wood Product 69(1): 37–40. doi: 10.1007/s00107-010-0421-2

Lukmandaru G. 2013. Antifungal activities of certain components of teak wood extractives. Jurnal Ilmu Teknologi Kayu Tropis 11(1): 11–18. doi: 10.51850/jitkt.v11i1.99

Masendra, Ashitani T., Takahashi K., Susanto M., Lukmandaru G. 2018. Lipophilic extractives of inner and outer barks from six different *Pinus* species grown in Indonesia. Journal of Forestry Research 29(3): 1329–1336. doi: 10.1007/s11676-017-0545-x

Masendra, Ngadianto A., Arifriana R., Maulidia F., Minorita W., Purba B.A.V., Lukmandaru G. 2021a. Polyphenol contents and free radical scavenging activity of *Pinus merkusii* cone. IOP Conference Series: Earth and Environmental Science 891, Indonesia, Sep. 2021. Mataram, 012017. doi: 10.1088/1755-1315/891/1/012017

Masendra, Purba B.A.V., Lukmandaru G. 2021b. Antioxidant activity of *Swietenia macrophylla* King bark extracts. Wood Research 66(1): 57–70. doi: 10.37763/wr.1336-

4561/66.1.5770

Masendra, Purba B.A.V., Lukmandaru G. 2021c. An evaluation of the antifungal and antioxidant activity of *Pinus merkusii* bark ethyl acetate extract. Jurnal Ilmu Kehutanan 15(1): 102–110. doi: 10.22146/jik.v15i1.1494

NIST11 2011. NIST/EPA/NIH Mass Spectral Database (NIST 11) and NIST Mass Spectral Search Program (Version 2.0g). National Institute of Standards and Technology, Gaithersburg, United States of America: 36–40. https://www.nist.gov/system/files/documents/srd/NIST1a11Ver2-0Man.pdf

Oliveira L.S., Santana A.L.B.D., Maranhão C.A., Miranda R.C.M., Lima V.L.A.G, Silva S.I., Nascimento M.S., Bieber L. 2010. Natural Resistance of Five Woods to *Phanerochaete chrysosporium* degradation. International Biodeterioration & Biodegradation 64(8): 711–715. doi: 10.1016/j.ibiod.2010.08.001

Pizzolitto R.P., Barberis C.L., Dambolena J.S., Herrera H.M., Zunino M.P., Magnoli C.E., Rubinstein H.R., Zygadlo J.A., Dalcero A.M. 2015. Inhibitory effect of natural phenolic compounds on *Aspergillus parasiticus* growth. Journal of Chemistry 2015(3), Article ID 547925. 7 p. doi: 10.1155/2015/547925

Preciado L.M., Lobo-Echeverri T., Pereaã±Ez J.A., Zopata K., Rojano B.A. 2016. Antioxidants from three *Swietenia* Species (Meliaceae). Journal of Medicinal Plants Research 10(2): 8–17. doi: 10.5897/JMPR2015.5967

Sekine N., Ashitani T., Murayama T., Shibutani S., Hattori S., Takahashi K. 2009. Bioactivity of latifolin and its derivatives against termites and fungi. Journal of Agriculture Food Chemistry 57(13): 5707–5712. doi: 10.1021/jf900719p

Srivastava B., Singh P., Shukla R., Dubey N.K. 2008. A novel combination of the essential oils of *Cinnamomum camphora* and *Alpinia galanga* in checking aflatoxin B1 production by a toxigenic strain of *Aspergillus flavus*. World Journal of Microbiology and Biotechnology 24(5): 693–697. doi: 10.1007/s11274-007-9526-0

Ulukanli Z., Karaborklu S., Bozok F., Ates B., Erdogan S., Cenet M., Karaaslan M.G. 2014. Chemical composition, antimicrobial, insecticidal, phytotoxic and antioxidant activities of Mediterranean *Pinus brutia* and *Pinus pinea* resin essential oils. Chinese Journal of Natural Medicines 12(12): 901–910. doi: 10.1016/S1875-5364(14)60133-3

Wijayanto A., Dumacay S., Gerardin-Charbonnier C., Sari R.K., Syafii W., Gerardin P. 2015. Phenolic and lipophilic extractives in *Pinus merkusii* Jungh. Et de Vries Knots and stem wood. Industrial Crops and Products 69: 466–471. doi: 10.1016/j.indcrop.2015.02.061

Xie Y., Wang Z., Huang Q., Zhang D. 2017. Antifungal activity of several essential oils and major components against wood-rot fungi. Industrial Crops & Products 108: 278–285. doi: 10.1016/j.indcrop.2017.06.041

Xu R.B., Yang X., Wang J., Zhao H.T., Lu W.H., Cui J., Cheng C.L., Zou, P., Huang W.W., Wang P., Li W.J., Hu X.L. 2012. Chemical Composition and Antioxidant Activities of Three Polysaccharide Fractions from Pine Cones. International Journal of Molecular Sciences 13(11): 14262–14277. doi: 10.3390/ijms131114262

Yang X., Zhao H.T., Wang J., Zhang H., Yao L., Zhang C.Y., Dong A.J., Ma Y., Wang Z.Y., Xu D.C., Ding Y. 2010. Chemical composition and antioxidant activity of essential oil of pine cones of *Pinus armandii* from the southwest region of China. Journal of Medical Plants Research 4(16): 1668–1672. doi: 10.5897/JMPR10.217

Yi J., Qu H., Wu Y., Wang Z., Wang L. 2017. Study on antitumor, antioxidant and immunoregulatory activities of the purified polyphenols from pinecone of *Pinus koraiensis* on tumor-bearing S180 mice in vivo International Journal of Biological Macromolecules 94(Pt A): 735–744. doi: 10.1016/j.ijbiomac.2016.10.071

Zabka M., Pavela R. 2013. Antifungal efficacy of some natural phenolic compounds against significant pathogenic and toxinogenic filamentous fungi. Chemosphere 93(6): 1051–1056. doi: 10.1016/j.chemosphere.2013.05.076

Zengin H., Baysal A.H. 2014. Antibacterial and

Antioxidant Activity of Essential Oil Terpenes against Pathogenic and Spoilage-Forming Bacteria and Cell Structure-Activity Relationships Evaluated by SEM Microscopy. Molecules 19(11): 17773–17798. doi: 10.3390/molecules191117773

Zhao Q., Qin J., Wang H., Wang J., Zhang X. 2019. Effects of different extraction methods on the properties of pine cone polysaccharides from *Pinus koraiensis*. BioResources 14(4): 9945–9956. doi: 10.15376/biores.14.4.9945-9956