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Antifungal Activities of Methanol Extract from *Syzygium chlorantha* and *Hopea* spp. against *Pycnoporus Sanguineus*

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Abstract

Antifungal activities of 6 methanol extract obtain from bark, sapwood and heartwood of *Syzygium chlorantha* and *Hopea* spp. were evaluated against the white rot fungi *Pycnoporus sanguineus* using a medium in which homogenised hyphae were dispersed. It is observed that *Hopea* spp. has antifungal activity against *Pycnoporus sanguineus* compared to *Syzygium chlorantha*. *Hopea* spp. bark showed the highest antifungal activity against *Pycnoporus sanguineus* with reaction showed very clear inhibition zone. Meanwhile, *Hopea* spp. sapwood showed partially clear inhibition zone. On the other hand, there are no antifungal activities occurring at heartwood of *Hopea* and all parts of *Syzygium chlorantha.* The positive control glycyrrhizic acid dipotassium salt (GADS) showed clear inhibition zone from 50 µg to 1000 µg. The results of the sapwood and bark of *Hopea* spp. suggest that these extract have great potential as a source of fungi stats.

1. Introduction

Malaysia has more than 100 species of timbers found in Peninsular Malaysia, Sabah and Sarawak. Timber is a wood that has excellent material property that has been used by mankind for thousands of years. It has superior performance as it is used for many purposes such as construction of buildings, furniture, bridges and wood products such as plows, hay rakes, wagons, spinning wheels and looms [1]. Referring to Ministry of Plantation Industries and Commodities Malaysia [2], the primary product that produce from Malaysian Timbers were saw logs, sawn timber, sleepers, veneer, chipboard, fireboard, fuel wood and plywood. Timber also was used as raw material for many industrial products such as paper mill, sawmill and even chemicals. Not only has that it acted as the major fuel source as people used it for heating, cooking and industry's fuel sources [1].

In scientific world, wood is categories as an organic material that mainly composed of cellulose, hemicelluloses and lignin [3]. According to Hill [4] timber is a renewable material that easily to degrade as all the chemical component will be broken down at the end of the life of a tree by biological organism. Biological organism is the major destroying agent such as fungi, bacteria, insects and marine borers [3]. This is the disadvantage characteristic of wood that used as services products. This phenomenon will cause the lifetime of timber shorter. Therefore, it is important to choose the wood that are stronger towards biological organism to make sure the products has reasonably long life in services [4].

Previous researches generally focus more on research timber such as cengal and merbau species [5] [6] [7]. If mankind keeps cutting same species of trees, these trees will be depleted faster as it takes a long time for the trees to grow and form mature period. Later on, they may not able to support our wood industry for the future. Out of 100 species that has been determined as Malaysian timber, there might have other species which have same or even more powerful characteristic and durability towards disease or fungal infection which suitable to replace the existing popular species which is cengal and merbau.

In this experiment, two species on Malaysian timber that were chosen are *Syzygium chlorantha* (Kelat Merah) and *Hopea spp*. (Merawan). Previous research showed that, there are limited experiment was conducted on antifungal activities on these two species. The objectives of this study was to know the fungi activities between *Syzygium chlorantha* and *Hopea* spp. two selected species of Malaysian timbers and to determine the antifungal activities of *Syzygium chlorantha* and *Hopea* spp. from Malaysian timbers species based on different parts.

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2. Materials and Methods

2.1. Plant Materials

Two species of commercial Malaysian timbers were selected which are *Syzygium chlorantha* (Kelat Merah) and *Hopea* spp. (Merawan). The timbers were obtained from Kandek Village, Dabong, Kelantan. The samples were separated into three parts which are bark, sapwood and heartwood. Each part was cut into small size forming a chip around 2-3 cm per each and were air-dried for 24 hours. Next, the samples were ground into sawdust with <1 mm in a grinder. The moisture content was determined by the following formula as shown in Equation (1).

$$\text{Moisture content (\%)} = \frac{\text{Air dried sample} - \text{Oven dried sample}}{\text{Air dried sample}} \times 100 \quad (1)$$

2.2. Extraction

The sawdust of each Malaysian timbers (1g) was extracted in a Soxhlet extractor with 30 ml of methanol. After that, all extracts were filtered before kept in bottle and stored in a fridge for further use. The same steps were repeated for other samples. The yield was measured for each sample of methanol extract with 4 ml in the oven at 105˚C 24 hours [8].

2.3. Antifungal Assay

The fungi strain that used in the experiment was white rot fungi of *Pycnoporus sanguineus* which provided by Universiti Sains Malaysia (USM). The fungi were incubated for 10 days in a PDA medium. Potato dextrose agar (PDA) medium was mixed with homogenized hyphae, and poured into the petri dishes. The paper discs were prepared by permeated with MeOH solution (10 µl for 5, 10, 20, 50, 100 µg/µl), glycyrrhizic acid dipotassium salt (GADS) as positive control and a disc without constituents as negative control. The paper disc was placed into the medium that contains PDA and homogenized hyphae. The discs were sealed and put in the incubator under 26˚C and 70% relative humidity.

It was observed after day 2, 3, 4 and so on, and their respective inhibition zones were observed. The results were expressed in terms of the clearness of the inhibition zone: -, inactive; +, partially clear inhibition zone; ++, clear inhibition zone; and +++, very clear inhibition zone. Determination of minimum inhibitory concentration (MIC) of the extract was defined as the lowest concentration that completely inhibited the growth for 24 hours [9]. The MIC for the *Syzygium chlorantha* and *Hopea* spp. extracts was determined by the agar well diffusion method.

3. Results and Discussion

3.1. Moisture Content

Moisture content of wood expresses as a percentage of its oven dry weight [10]. Figure 1 shows the percentage moisture content of *Syzygium chlorantha* and *Hopea* spp. based on different parts of bark, sapwood and heartwood.

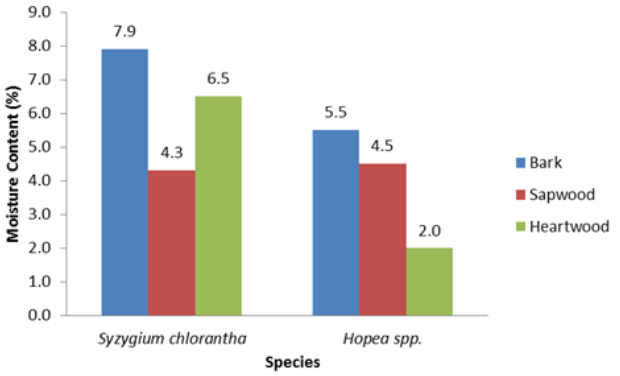

Figure 1: Percentage moisture content of *Syzygium chlorantha* (Kelat Merah) and *Hope* spp. (Merawan) based on different parts.

Based on Figure 1, it is identified that both species has bark as the highest percentage of moisture content. This result was probably due to its characteristic and function to protect the timber, help in transportation in water and help in respiration process which causes it to have high moisture content [11].

However, the percentage moisture content of sapwood and heartwood of *Syzygium chlorantha* and *Hopea* spp. has different pattern. In *Syzygium chlorantha*, the heartwood has more percentage of moisture content than sapwood while in *Hopea spp*., it is vice versa.

Moisture content of sapwood was higher than heartwood probably due to its primary role in conducting water from the root to the crown [12], serves as water storage and release water for transpiration process [11]. Meanwhile, Ogunwusi [12] mention that sapwood act as a site for living cells that can produce of more tissues or defensive compounds when respond to injury.

On the other hand, researched by Goranson [11] towards white oak found that heartwood was used as extra storage area for water, where it soaking in water when there is extra water availability in the sapwood and release the water back to the sapwood during water stress period. This means that the extra water at sapwood of *Syzygium chlorantha* had soaked in at the heartwood. Besides that, Casseens [13] identify that sapwood usually dry faster than heartwood. Heartwood is dried slowly due to the availability of extractives or other obstruction which inhibit drying. This might be the reason more extra water availability or moisture content can be determined at *Syzygium chlorantha* heartwood, where its acts as the extra storage of water and were slow in drying process.

3.2. Yield Extractive

Figure 2 shows and differentiates the yield extractive among *Syzygium chlorantha* and *Hopea* spp.

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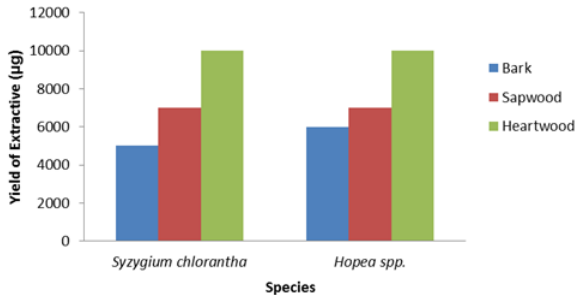

Figure 2: Comparison the yield extractive among *Syzygium chlorantha* (Kelat Merah) and *Hopea* spp. (Merawan).

Referring to Figure 2, it is clearly identified that the heartwood contains the highest yield extractive followed by sapwood and bark for both of *Syzygium chlorantha* and *Hopea* spp.

Researched by Morais and Pereira [14] found that heartwood contain high amount of xylem. Taylor et al. [15] also identified that the extractive of heartwood might also contain nonstructural wood components. It also has combination of compounds present in the adjacent sapwood and materials imported from the phloem. This is the reason why heartwood of both species *Syzygium chlorantha* and *Hopea* spp. contain high yield extractive.

Meanwhile, the yield extractive of sapwood was higher than bark for *Syzygium chlorantha* and *Hopea* spp. probably due to the higher amount of lipids and starch that can be found in sapwood compare to amount of lignin that contain in the bark. According to Ogunwusi [12], based on studied by Kawamura et al. [7] found that sapwood contains many component such as lipid and starch which composes of living cell carbohydrate, glucose, sucrose, fructose and cellulose. Besides that, sapwood was also responsible for storage and synthesis of biochemical.

3.3. Antifungal Activity

The result of the antifungal activities of methanol extracts from bark, sapwood and heartwood of two selected Malaysian timber which are *Syzygium chlorantha* and *Hopea* spp. against white-rot fungus, *Pycnoporus sanguineus* are summarized in Table 1. The *Hopea* spp. shows an antifungal activity against *P. sanguineus* compared to *Syzygium chlorantha*. Bark from *Hopea* spp. showed the highest antifungal activity against *Pycnoporus sanguineus* with a very clear inhibition zone. Sapwood of *Hopea* spp. also showed reaction against *Pycnoporus sanguineus* with partially clear inhibition.

The minimum of effective amounts of methanol extract from bark and sapwood of *Hopea* spp. both were 500 µg. Meanwhile, the positive control glycyrrhizic acid dipotassium salt (GADS) showed clear inhibition zone from concentration 50 µg to 1000 µg. The difference inhibition zone activities of *Syzygium chlorantha* and *Hopea* spp. on different parts might due to the chemical composition, their function and activity of *Pycnoporus sanguineus.*

Table 1: Antifungal activities of the methanol extracts from *Syzygium chlorantha* (Kelat Merah) and *Hopea* spp. (Merawan) selected Malaysian timber species against *Pycnoporus sanguineus.*

Species	Parts	Inhibition zone[a]				
		Pycnoporus sanguineus				
		1000 µg	*500 µg*	*200 µg*	*100 µg*	*50 µg*
Syzygium chlorantha	Bark	-	-	-	-	-
	Sapwood	-	-	-	-	-
	Heartwood	-	-	-	-	-
Hopea spp.	Bark	+++	+++	-	-	-
	Sapwood	+	+	-	-	-
	Heartwood	-	-	-	-	-
GADS[b]		++	++	++	++	++
Paper disc[c]		-	-	-	-	-

[a]: Inhibition zone of (+++) showed very clear inhibition zone; (++) showed clear inhibition zone; and (+) showed partial inhibition zone.
Inhibition zone that same as negative control or no inhibition zone were expressed as no inhibition (-). Test were carried out in triplicate.
[b]: Positive control, glycyrrhizic acid dipotassium salt (GADS).
[c]: Negative control

The Figures 3 show the antifungal activity of *Syzygium chlorantha* against *Pycnoporus sanguineus* based on different concentration. It is clearly observed that there are weak or no inhibition zone on every concentration from 50 µg to 1000 µg of bark, sapwood and heartwood of methanol *Syzygium chlorantha* extractives. The extract of these species appeared to contain large amounts of less active antifungal constituents.

The weak or no antifungal activities against *Pycnoporus sanguineus.* in bark was probably due to the less number of lignin component which content phenolic content which specifically act as antioxidant and antimicrobial characteristic [16] [17] and macromolecular and heterogeneous polymers which increase the difficulty of lignin to be degraded [18].

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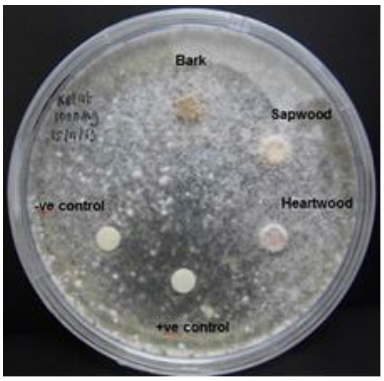

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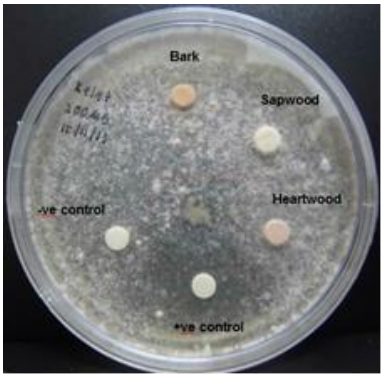

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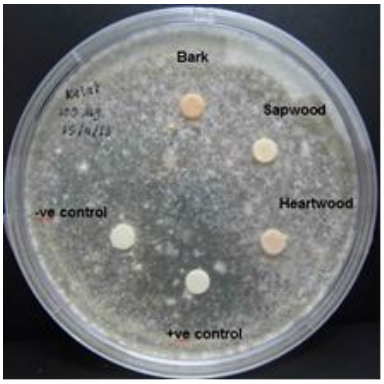

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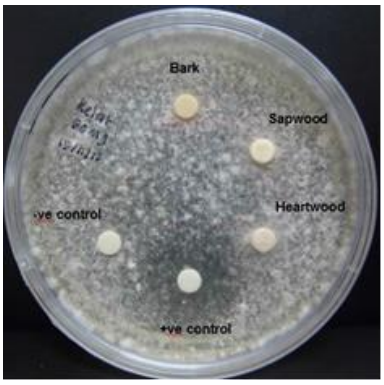

(e)

Figure 3: The antifungal activity of *Syzygium chlorantha* against *P. sanguineus* at concentration (a) 1000 µg, (b) 500 µg, (c) 200 µg, (d) 100 µg and (e) 50 µg.

Meanwhile, the less number of living cells such as carbohydrate, glucose, sucrose, fructose and cellulose in sapwood was probably the reason it did not showed antifungal activity [12]. Cellulose is a chain of glucose linked by ß-1,4-glycosidic bond to form polysaccharide [16]. Due to less number of this polysaccharide linkage causes the *Pycnoporus sanguineus* easily in breaking the structure of cellulose and attacked the sapwood of *Syzygium chlorantha.*

Not only that, the heartwood of *Syzygium chlorantha* also did not show any antifungal activity even though the yield concentration was high. This is probably because the extractives that contain in the heartwood mostly are from inactive and nonstructural wood component. Heartwood is the dead part of tree since it is located at the innest part and did not provide structural strength to the timber [19] [15].

The Figures 4 show the antifungal activity of *Syzygium chlorantha* against *Pycnoporus sanguineus* based on different concentration.

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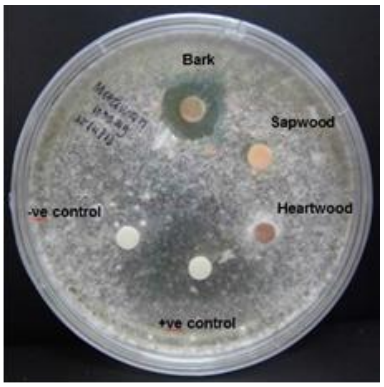

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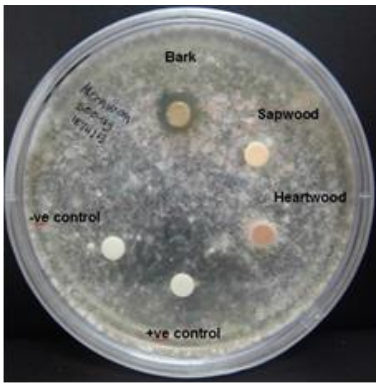

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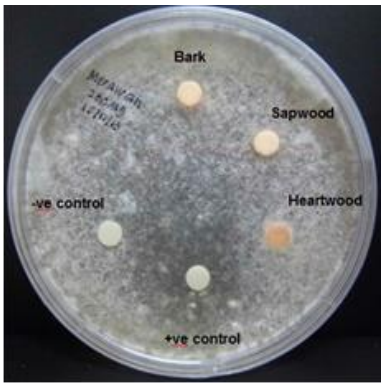

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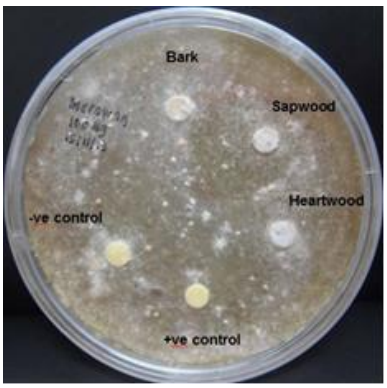

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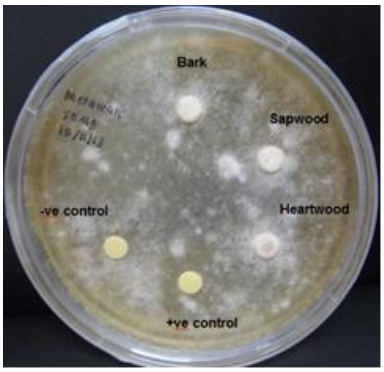

(e)

Figure 4: The antifungal activity of *Hopea* spp. against *Pycnoporus sanguineus* at concentration (a) 1000 µg, (b) 500 µg, (c) 200 µg, (d) 100 µg and (e) 50 µg.

Based on Figure 4, it was observed that only bark and sapwood of *Hopea* spp. extract give positive antifungal activities against *Pycnoporus sanguineus*. There are several reasons to prove bark has highest antifungal activity compared to sapwood and heartwood. Researched by U.S. Department of Energy [20] stated that wood bark has high lignin content. According to Jinjing [16], lignin polymers contain many active groups and one of it is phenolic hydroxyl groups. The phenolic contains specifically function as natural antioxidant [17] and possess antimicrobial characteristic, which help plants defense against fungi. To summarize, the *Hopea* spp. might contain high amount of lignin component which contain active groups such as phenolic content that act as antioxidant and antifungal activity against *Pycnoporus sanguineus.*

Sapwood of *Hopea* spp. also generates antifungal activity towards *Pycnoporus sanguineus* as it showed partially clear inhibition as shown in Figure 5. This means that, the reaction is not strong as bark of *Hopea* spp. According to Saranpää and Höll [21], sapwood stored metabolites such as lipids and starch. Lipids are naturally occurring organic compound or molecule that contain hydrocarbon and make up the structure of building blocks and function of living cells. Starch is a naturally abundant nutrient of carbohydrate. Researched by Jinjing [16] determined that the main constituent of wood carbohydrate is cellulose, a chain of glucose units joined to form polysaccharide by ß-1, 4-glycosidic bond linkage. Cellulose is a major component in the structure of plant and insoluble in most solvents including strong alkali which difficult to separate it from wood and eventually closely integrated with lignin and hemicelluloses. It can be summarize that due to the structure of sapwood, it is hard for *Pycnoporus sanguineus* to break the polysaccharide linkage of carbohydrate.

According to Käärik [22], mention that sapwood is usually destroyed more quickly than heartwood and it is found that only heartwood has the strong natural

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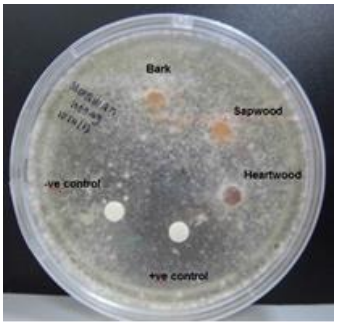
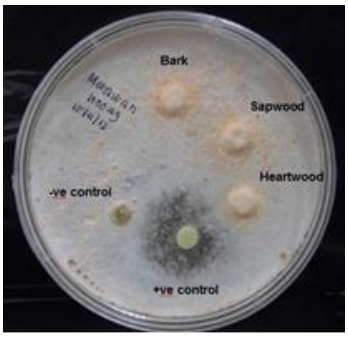
resistance to fungi or other destructive organism. However, based on this experiment, it is observed that the bark and sapwood are more resistant toward *Pycnoporus sanguineus* fungi than heartwood. This might be due to the decreasing amount of glucose, sucrose and fructose in heartwood compare to sapwood which weaken the structure of the cell. Meanwhile, in some timber, it is reported that the amount of starch disappear from the plastids of the ray parenchyma cells during formation of heartwood [21].

It can be concluded that the component that contain mostly in bark is lignin, which the lignin polymerase contain active groups such as phenolic content than can act as antioxidant and antifungal activities. Meanwhile, sapwood contains metabolites such as lipids and starch that responsible in structuring the cell.



Day 7



Day 15

Figure 5: Antifungal activity of extractive *Hopea* spp. (Merawan) at 1000 µg in day 7 and day 15.

In this researched, it is determined that the extractive *Hopea* spp. at concentration 1000 µg and 500 µg showed antifungal activities in day 4 and day 5 after incubation. However in day 7, the fungi start to cover the paper disc of bark and sapwood *Hopea* spp. Meanwhile, for GADS the fungi only start to cover the paper disc after day 15 in the incubator.

There are several reasons reaction of bark and sapwood of *Hopea* spp. occur only in short period compared to positive control (GADS). This is because, *P. sanguineus* is a fungi group of Basidiomycetes which specialized in wood degradation [23], and capable in degrading all cell wall components which are cellulose, lignin and hemicelluloses of wood [3] and the most persuasive fungi in degrade the holo-cellulose component [24].

Pycnoporus sanguineus is fungi that able to degrade the bark of timber. According to Castaňeda et al. [23], based on previous research of Perez-Jimenez [25] and Martínez et al. [26] stated that white rot fungi have capacity to exploit all wood components due to the secretion of a variety of lignocellulolytic enzymes. This statement supported by Eugenia et al. [27], which determined that *P. sanguineus* is a white-rot fungi which produce ligninolytic enzyme such as laccases. These laccases able to endure temperature as high as 60˚C and useful in phenolic degradation, pulp bleaching and dye decolorization. Researched by Gusse *et al.* [28] also identified that white-rot fungi have evolves to produce a very powerful and nonspecific bank of enzymes called ligninases that degrade lignin.

Pycnoporus sanguineus also able to degrade cellulose of sapwood. This is because *Pycnoporus sanguineus* which are under Basidiomycetes are the most potent degraders towards cellulose because they grow on the dead wood or litter, in environment rich in cellulose. Basidiomycetes also utilize a set of hydrolytic enzyme typically composed of endoglucanasc, cellobiohydrolase and ß-glucosidase [24].

This mean that, even though bark and sapwood contain lignin and phenolic content than can act as antioxidant and antifungal activities and cellulose that have strong bonding, but the strength is not too strong to against *Pycnoporus sanguineus* which has lignocellulolytic enzyme and ligninases that can degrade the lignin and phenolic; and hydrolytic enzyme that can degrade cellulose.

4. Conclusion

Hopea spp. has antifungal activity compared to *Syzygium chlorantha*. *Hopea* spp. bark showed the highest antifungal activity, followed by *Hopea* spp. sapwood against *P. sanguineus* with both have minimum effective amount of 500 µg. These extracts which showed antifungal activities without any fractionation or purification suggest that they have great potential as a source of fungistats. Meanwhile, the bark of *Hopea* spp. *and Syzygium chlorantha* wood which did not possess any antifungal activity indicates that treatment of wood is a must in the wood manufacturing products. The properly wood treatment will ensure the durability of wood from being attack by fungi.

References

[1] Bowyer, J.L., Shmulsky, R., and Haygreen, J.G. (2007). Forest products and wood science. Blackwell. Iowa. p. 3-164.
[2] Ministry of Plantation Industries and Commodities Malaysia (2009). National Timber Industry Policy (NATIP) 2009-2020.
[3] Haque, N. (1997). White rot fungal decay of wood. 18p.
[4] Hill, C. (2006). Wood modification: Chemical, Thermal and Other Processes. John Wiley and Son Ltd. Bangor. p. 1- 44.

eISSN Number: 2462-2389

[5] Takahashi, M. and Kishima, T. (1973). Decay resistance of sixty-five Southeast Asia timber specimens in accelerated laboratory tests. p. 525-541.

[6] Kishino, M., Ohi, H., and Yamaguchi, A. (1995). Characteristics of methanol extractives from chengal wood and their antifungal properties. 41, p. 444-447.

[7] Kawamura, F., Ramle, S.F.M., Sulaiman, O., Hashim, R., and Ohara, S. (2010). Antioxidant and antifungal activities of extracts from 15 selected hardwood species of Malaysian timber. Journal of Wood Product, 6p.doi:10.1007/s00107-010-0413-2.

[8] Kawamura, F., Shaharuddin, N. A., Sulaiman, O., Hashim, R., and Ohara, S. (2010). Evaluation on antioxidant activity, antifungal activity and total phenols of 11 selected commercial Malaysian timber species. 44(3), p. 319-324.

[9] Thongson, C., Davidson, P.M., Mahakarrchanakul, W. and Weiss, J. 2004. Antimicrobial activity of ultrasound assisted solvent extracted spices. Letters in Applied Microbiology 39: 401-406.

[10] Eckelman, C.A. (2003). Textbook of product engineering and strength design of furniture, Purdue Univ., West Lafayette, Indiana.

[11] Goranson, C. E. (2005). Thesis degree: Whole-stem water reltions in white oak (*Quercus alba L.*). Georgia.

[12] Ogunwusi A. A. (2013). Heartwood, sapwood and bark proportions in five lesser used tropical hardwood species growing in Nigeria. Journal of Biology, Agriculture and Healthcare 3(1), p. 93-99.

[13] Casseens, D. L. (1914). Top weight estimates for sawlog size hardwood lumber. Retrieved from http://www.extension.purdue.edu/extmedia/FNR/FNR-109.html

[14] Morais, M. C., and Pereira, H. (2012). Variation of extractives content in heartwood and sapwood of *Eucalyptus globulus* trees. Journal wood science and technology, 46(4), p. 709-719.

[15] Taylor, A. M., Brooks, J. R., Lachenbruch, B. and Morrell, J. J. (2007). Radial patterns of carbon isotopes in the xylem extractives and cellulose of Douglas-fir. p. 921-927.

[16] Jinjing, L. (2011). Bachelor's thesis: Isolation of lignin from wood. 57 p.

[17] Ibrahim, N. M., Mat, I., Lim, V., and Ahmad, R. (2013). Antioxidant activity and phenolic content of *Streblus asper* leaves from various drying methods. 2, p. 156-166 doi:10.3390/antiox2030156

[18] Hatakka, A. and Hammel, K. E. (2010). Fungal Biodegradation of Lignocelluloses. p. 319-340.

[19] Forest Academy. Annual growth ring. Retrieved November 2013 from https://www.theforestacademy.com/tree-knowledge/annual-growth-rings/

[20] US Department of Energy. (2004). Wood adhesives from bark-derived phenol. Retrieved from http://www1.eere.energy.gov/bioenergy/fy04/wood_adhesives.pdf

[21] Saranpää, P. and Höll, W. (1989). Soluble carbohydrates of *Pinus sylvestris* L. sapwood and heartwood, 3(3) p. 138-143.

[22] Käärik, A. A. (1974). Decomposition of wood. Dickinson C. H., and Pugh, G.J.F., (Eds), *Biology of Plant Litter Decomposition, Volume* 1, (p. 129-169). London and New York. Harcourt Brace Jovanovich.

[23] Castañeda, R. E. Q., López, E. B., González, E. D., Martinez, A., Mallol J. F., and Anaya C. M. (2009). Characterization of cellulolytic activities of *Bjerkandera adusta* and *Pycnoporus sanguineus* on solid wheat straw medium. Journal of Biotechnology, 12(4) 3p.

[24] Baldrian, P. and Valaskova, V. (2008). Degradation of cellulose by basidiomycetous fungi. 32, p. 501-521.

[25] Perez-Jimenez., R.M. (2006). A review of the biology and pathogenicity of Rosellinia necatrix – the cause of white rot disease of fruit trees and other plants. Journal of Phytopathology, 154, 257-266.

[26] Martinez, D., Gil, R., Slifstein, M., Hwang, D. R., Huang, Y., Perez, A., et al. (2005). Alcohol dependence is associated with blunted dopamine transmission in the ventral striatum. Biol. Psychiatry 58, 779–786. doi: 10.1016/j.biopsych.2005.04.044

[27] Eugenio, M. E., Carbajo, J. M., González, A. E., and Villar, J. C. (2009). Laccase production by *Pynoporus sanguineus* under different culture conditions. Journal of Basic Microbial, 49(5) p. 433-440, doi: 10.1002/jobm.200800347.

[28] Gusse, A. C., Miller, P. D. and Volk T. J. (2006). White-rot fungi demonstrate first biodegradation of phenolic resin. Environment Science Technology, 40(10) p. 4196Kishino, M., Ohi, H., and Yamaguchi, A. (1995). Characteristics of methanol extractives from chengal wood and their antifungal properties. 41, p. 444-447.

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