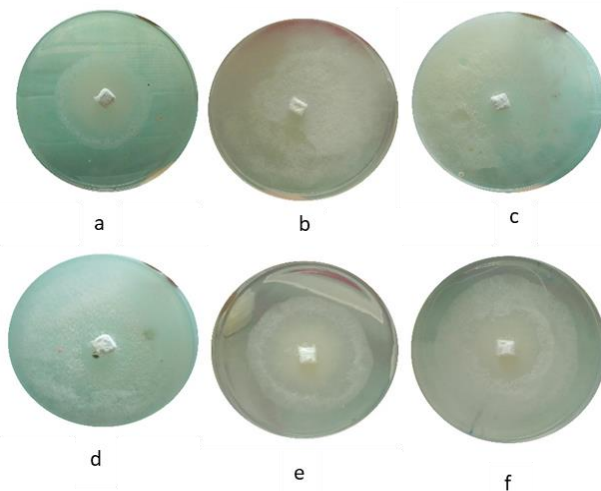
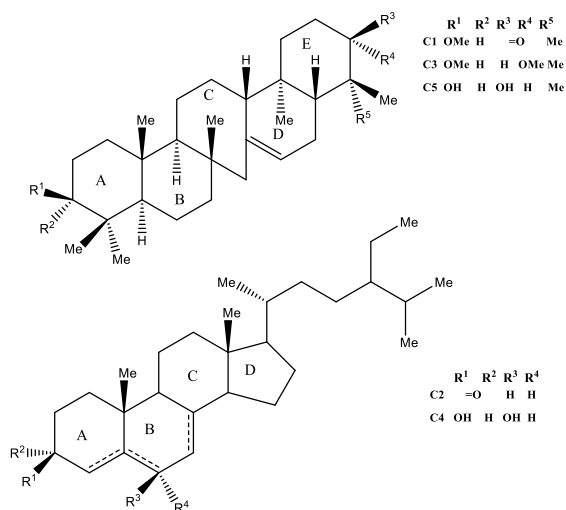


# WOOD RESEARCH Journal

## Journal of Indonesian Wood Research Society

Volume 11, Number 2, October 2020



- Wood Permeability Assessment of Young Teak (*Tectona grandis* L.f.)
- Improving Fire Resistance of Mahogany (*Swietenia macrophylla*) Wood Impregnated with Mixture of Borax and Boric Acid
- Strength and Stiffness Behavior of Earthquake Resistant Pedestrian LVL Timber Bridge
- Reduction in Toxicity of Tectoquinone Against *Reticulitermes speratus* Kolbe Termites
- Antifungal Activity of Triterpenoids and Steroids Isolated from *Pinus merkusii* Bark Against *Phanerochaete chrysosporium*

**Ratih Damayanti**, Krisdianto, Jugo Ilic, Gustan Pari, Peter Vinden, and Barbara Ozarska 41

**Tomy Listyanto**, Aditya Aji Pratama, Keisuke Ando, and Nobuaki Hattori 48

**Bernardinus Herbudiman**, Delima, and Yosafat Aji Pranata 53

**Ganis Lukmandaru** 58

**Masendra**, Brandon Aristo Verick Purba, and Ganis Lukmandaru 65

# WOOD RESEARCH Journal

## Journal of Indonesian Wood Research Society

### Chief Editor

Prof. Wahyu Dwianto

### Editorial Board Members

Prof. Yusuf Sudo Hadi  
Prof. Bambang Subiyanto  
Prof. Wasrin Syafii  
Prof. Anita Firmanti  
Dr. Joko Sulistyono

Prof. Nobuaki Hattori  
Prof. Jugo Ilic  
Prof. Edi Suhaimi Bakar  
Prof. Sri Nugroho Marsoem  
Prof. Imam Wahyudi

Dr. Krisdianto  
Dr. Ganis Lukmandaru  
Dr. Tomy Listyanto  
Dr. Ratih Damayanti  
Fanny Hidayati, Ph.D.

### Peer Reviewers

*Indonesian Institute of Sciences*  
Prof. Subyakto  
Prof. Myrtha Karina  
Dr. Puspita Lisdiyanti

*Bogor Agricultural University*  
Prof. Fauzi Febrianto  
Prof. I Wayan Darmawan  
Dr. Naresworo Nugroho

*Gajah Mada University*  
Dr. Ragil Widyorini

*Mulawarman University*  
Dr. Rudianto Amirta  
Dr. Irawan W. Kusuma

*Hasanuddin University*  
Prof. Musrizal Muin

*Tanjungpura University*  
Prof. Yuliati Indrayani

*Kyoto University*  
Prof. Junji Sugiyama  
Prof. Toshiaki Umezawa  
Prof. Takashi Watanabe

*Dresden University*  
Dr. Christian Gottlöber

*Oklahoma State University*  
Prof. Salim Hiziroglu

*Montpelier University*  
Prof. Joseph P. Grill

*Duzce University*  
Prof. Cihat Tascioglu, Ph.D

*Kangwon National University*  
Prof. Nam-Hun Kim

*University of Melbourne*  
Prof. Barbara Ozarska

*Paris Tech Cluny*  
Prof. Remy Marchal

*Universiti Putra Malaysia*  
Prof. Edi Suhaimi Bakar

### Journal Manager

Dr. Ratih Damayanti

### IT Manager

Suhardi Mardiansyah, A.Md.

### Section Editors &

Dr. Ratih Damayanti,

### Initial Reviewers

Fanny Hidayati, Ph.D.

Dian Anggraini Indrawan, S.Hut., M.M.

### Copy & Layout Editor

Suhardi Mardiansyah, A.Md.

### Proof Readers

Prof. Subyakto

Dr. Krisdianto

Dr. Tomy Listyanto

### Treasurer

Dr. Firda Aulya Syamani, M.Si.

# Wood Permeability Assessment of Young Teak (*Tectona grandis* L.f.)

Ratih Damayanti, Krisdianto, Jugo Ilic, Gustan Pari, Peter Vinden, and Barbara Ozarska

## Abstract

Wood properties of young teak (*Tectona grandis* L.f.) is inferior, and then preservative treatment is one possible solution to enhance its service life. The uptake and movement of preservatives through wood cell structure is directly connected to the wood permeability. There are two simple methods to identify wood permeability: water soaking and bubble test methods. This paper assesses the young teak permeability by water soaking and bubble test methods. The assessment was conducted on five cm thick young-teak discs by soaking in the red-dye water and blowing air into the discs which had been coated with soap. Results show that the heartwood is less permeable than sapwood. Red-dye penetrates almost 100% of the sapwood area, and the red-dye did not penetrate in the heartwood. The red-dye only penetrates in the cracked heartwood through the void volume in the cracking heartwood. There is a transition zone between sapwood and heartwood, and it is refractory. Bubble test with air pressure from compressor could open the air-pathway in the heartwood and sapwood of young-teak discs taken from Bogor (wet site). The bubble test result of young-teak discs from Madiun (dry site) showed air-pathway only in the sapwood, but not in the heartwood. The air pressure is not capable of moving the vapour through the wood cell. It indicates that the heartwood of young-teak from Madiun is less permeable and less possibility for pressure treatment.

**Keywords:** Young-teak, permeability, water soaking, bubble test, heartwood, sapwood, transition zones.

## Introduction

Teak wood (*Tectona grandis* Linn. f) is one of the most valuable woods for the construction of boats, furniture, flooring, decorative objects, and decorative veneer. However, the use is limited due to scarcity and high cost. Mature teak heartwood has excellent dimensional stability and a very high degree of natural durability. In general, teak is worked with moderate ease with hand and machine tools (Falk 2010). Mature teak is mostly harvested from the plantation at the age of 50-80 years old in India and Indonesia (Soerianegara and Lemmens 1994). This long rotation has caused the price of teak wood to increase significantly due to a limited supply. Iskak (2005) stated that the shortage of teak as a raw material had been estimated at approximately 2 million m<sup>3</sup> per year. Consequently, timber industries that rely on teak as raw material face difficulties in its continuity of supply (Krisdianto and Sumarni 2006).

This situation motivates the silviculturists to investigate various methods which would allow establishing a shorter rotation and a faster growth of teak. One of the methods already developed is through vegetative cultivation, such as tissue culture, bud grafting and shoot cutting. As a result, the rotation age can decrease from 50-80 years to 20-40 years (Yunianti 2012). Fast-growing tree with high productivity forest plantations is becoming an essential supply of wood in the tropics, especially in the countries where agricultural and forest sectors support the economy. Increasing wood production is important since the commercial dimensions are reached in a relatively short period (Kanninen 2003). However, this intensive timber production may induce alteration in the anatomical and technical properties, and as a result, the suitability of the

wood for high-quality products is reducing (Saranpaa 2003). In Indonesia, many varieties of fast-growing teak have been widely cultivated. Timber communities call this timber "super teak". One of them is *Jati Utama Nasional* (JUN). The combination of breeding technology and intensive silviculture treatment has enabled teak timber producers to harvest the tree at a very young age of 5 years old.

The inferior characteristic of young teak could be overcome by preserving timber in the means of penetrating preservatives into the timber, to improve its service life (Lebow 2010). Preservative treated wood can be protected from the attack of decay fungi, harmful insects, or marine borers. The uptake and movement of preservatives through the wood cell structure is directly connected to the wood permeability. In 2007, Kamke and Lee mentioned that permeability is physical properties of wood related to the ease with which fluids are transported through a porous under differential pressure, indicating the magnitude liquid flow in the material and varying factors such as chemical and anatomical properties, flow direction and type of fluid. Ahmad and Chun (2009) and Pokki *et al.* (2009) stated that wood permeability is of great variability due to structure and vessels orientation, which facilitates longitudinal flow and play a major role in the movement of liquids.

There is a variation of wood permeability radially. Liquid penetration into the wood cell varies according to the wood cell component, shapes, and cell wall content, which allows liquid penetration through diffusion. Heartwood being less permeable, showed a reduction in the volume of vapour that moved through the wood as well as much lower moisture content compared to the sapwood. Between heartwood and sapwood, there is a transition zone which is claimed as refractory. Barnacle and Ampong initially studied

the wood transition zone in 1974. Hillis (1987) mentioned that the transition zone is: *“a narrow, pale-coloured zone surrounding some heartwoods and injured regions, often containing living cells, usually devoid of starch, often impermeable to liquids, with a moisture content lower than the sapwood and sometimes also the heartwood.”* The state of permeability variation is obvious. This paper studies the permeability of young teak wood transversely by observing young-teak disc by a simple method of water soaking and bubble soap.

## Materials and Methods

### Sample Preparation

Twenty 5-year old fast grown teak discs were collected from Bogor area (A) and Madiun area (B). The disc collected from Bogor area represented the teak which has been planted in the wet area, while disc from Madiun area known as teak from the dry area. Five cm thick discs (14-29 cm in diameter) were cut from the breast-high teak tree and grouped into water soaking and bubble soap methods. Both disc surfaces were sanded by 150-grit sandpaper followed by 400-grit sandpaper for uniformity. The discs were then air-sprayed to clean up the sawdust and dirt on the surfaces.

### Water Soaking Method

Twenty-five ml red-food-dye was diluted into a litre of aquadest in the plastic container. Teak wood discs were

then soaked in upper-side one by one for four days. Red-dye water movements of the wick action from the bottom part into the upper part were identified carefully.

### Bubble Test Method

On the upper part of the disc, liquid soap was coated in the 4 cm width line across the diameter through the pith to the bark. Parallel with the line through the pith on the bottom part of the disc, and the air was blown through the air compressor, which set into 10 bar. The bubble on the upper surfaces shows the blown soap indicates the flowing pattern longitudinally.

### Results and Discussion

Water soaking method showed red-dye penetrated to all of the sapwood portion of the discs and not the heartwood. Figure 1 shows red-dye penetrated in the sapwood and little crack of the heartwood.

The wick action of heartwood and sapwood part of the discs were investigated with distinct moisture distribution, and behaviours found. Heartwood, being less permeable, showed no red-dye penetration compared to the sapwood. The outer portion of the tree known as sapwood is living cell, the inner sapwood zone matures and dies as the wood ages.

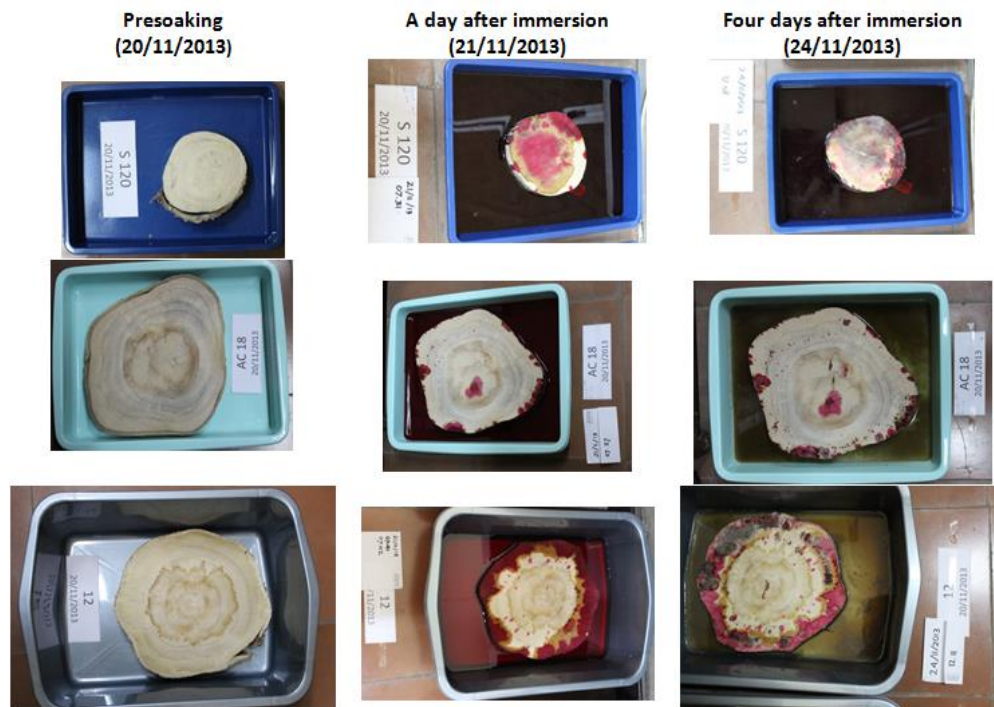


Figure 1. Red-dye penetration of the young-teak discs.

During the maturation process, extractives and other extraneous chemicals begin to precipitate in the pits and other fine pores of the lumen (Stamm 1964; Butterfield and Meylan 1980). The build-up of this material in the older sapwood alters various properties of the wood, including colour, permeability, density and durability (Kollman and Cote 1968). The dark non-conducting inner wood is known as heartwood, which could be 100 times less permeable than sapwood from the same tree. The permeability change is usually gradual, with the inner wood being the least permeable. Diffusion is not limited by permeability, with the diffusion coefficient of heartwood being only slightly less than the corresponding diffusion coefficient of sapwood (Stamm 1964).

In teak wood, there is transition zone lays between sapwood and heartwood and it is known as the refractory zone, which may raise problems in preservative treatments in teak wood (Barnacle and Ampong 1974) and similarly found in poplar (Murphy *et al.* 1991). Barnacle and Ampong (1974) observed preservative problems associated with the occurrence of a relatively wide zone of intermediate wood nearly impermeable to treatment in fence posts cut from 15 cm in diameter of 15-year old un-pruned plantation-grown *Tectona grandis* trees, and alternating penetrated. Non-penetrated bands of heartwood in some preservative-treated small fence posts. The characteristics of this zone were pale in colour, in contrast to sapwood, about 1.5 cm in

width, free from starch, impermeable, and containing tyloses. This phenomenon was also observed surrounding the heartwood of *Manilkara multinervis*. Furthermore, observations made at the Forest Products Research Institute (FPRI), Kumasi, indicated that intermediate wood is a common feature in fence post of teak from at least one district in Ghana and that it is generally impermeable to treatment even at a pressure of 200 lb in<sup>-2</sup> (Barnacle and Ampong 1974).

The same phenomenon was also observed in another hardwood genus, poplar. In the course of study on the CCA treatment and durability of poplar timber, a zone of refractory wood at the sapwood/heartwood boundary was observed. The most striking feature of CCA in *Poplar tacamahaca x trichocarpa* 32 was the observation of an impermeable zone, generally consisting of two growth rings, at the boundary of the sapwood and heartwood (Murphy *et al.* 1991). Furthermore, in 2012, Norton conducted a vacuum pressure impregnation using copper-based preservative system (CCA and Copper Naphthenate ) on six and a half year old of teak containing sapwood and heartwood obtained from tropical north Queensland Australia. The result showed that from the six specimens, the outer zones of the sapwood were fully penetrated and the heartwood remained un-penetrated. The transition zone is apparent in samples 9, 10 and 12 and is not penetrated (Figure 2).



Figure 2. CCA treated test specimen from Australia. (Source: Norton 2012).

The part at least of the relatively white or colourless appearance of the transition zone is due to a moisture content that is lower than that of the adjacent sapwood and sometimes the heartwood. In *Taxus baccata* felled in late autumn, the moisture content (MC) of the transition zone (50%) was less than that of the heartwood (100%) and the sapwood (130%) (Craib 1923 in Hillis 1987). Yazawa and Ishida (1965b in Hillis 1987) found a similar but less marked contrast with *Sorbus*, *Acer*, *Cornus*, *Magnolia*,

*Acanthopanax*, *Ocroya*, *Tilia*, and *Quercus* species. In *Pinus radiata*, the MC of the transition zone is similar to that of the heartwood (about 40% on a dry weight basis) compared with 160% for the surrounding sapwood (Shain and Mackay 1973a in Hillis 1987).

The width of the transition zone usually about 1-3 growth rings, can vary seasonally, that depends on site and climatic conditions (Shain and Mackay 1973a in Hillis 1987). Samples were taken at increasing heights in the tree reach

a level where heartwood is no longer present, and only the transition zone and sapwood exist (Hillis 1987). In irrigated and non-irrigated specimens of various provenances of the latter species, the transition zone had the same width of almost one growth ring (Polge 1982 in Hillis 1987). Different to Shain and Mackay (1973a in Hillis 1987), J.M. Harris (personal communication in Hillis (1987) observed a transient region of transition zone up to about 60 mm thickness within the dry wood zone of *Pinus radiata* which was present at all times. The transition zone of *P. radiata* grown in New Zealand first become evident in late winter at a point some distance up the tree extends upward and downward in the tree during early spring. It disappears in late spring and early summer, presumably with the formation of heartwood (Harris 1954 in Hillis 1987). With the same species are grown in Australia, a dry transition zone was observed in all trees examined throughout the year (Shain and Mackay 1973 in Hillis 1987).

Transition zones occur around mechanical injuries, as in *Sorbus alnifolia* (Yazawa and Ishida, 1965b in Hillis 1987), or the wounds formed by *Sirex noctilio* in *Pinus radiata* and around the necrotic, phenol-enriched sapwood of *Picea abies* affected by *Formes annosus*. Usually, the transition zone is up to 3 mm wide in *Pinus radiata* and with a width of less than one growth ring, up to 7 mm wide in *Eucalyptus* species and a width of less than two growth ring and *Cryptomeria japonica* can have a width of 10-18 mm containing 4-10 growth rings (Nobuchi and Harada 1983 in Hillis 1987).

In some species, the dark discolouration can be found in the heartwood boundary that is the third type of stain formed during the transition of sapwood into the heartwood. Two other types of dark discolouration are found in fully functional sapwood as a result of injury, and the second is found in normally coloured fully mature heartwood. Sachs et al. (1966 in Hillis 1987) observed these three types of dark discolouration in the wood of living *Quercus*. The specific gravity of the sapwood-heartwood boundary stain is greater than either the adjacent sapwood or heartwood and its ash content is lower than the adjacent sapwood. Still, there are more copious amounts of dark-coloured substances than in normal heartwood. The distribution of these substances varies from surrounding the annual rings to other confined to one quadrant of the stem.

Longitudinally, their length varies from one or two meters to the entire length of the stem connecting a large crown branch with a major root (Hillis 1987; Murphy et al. 1991). Almost all the tree examined (Bulgrin and Ward 1968 in Hillis 1987) which contained sapwood-heartwood boundary stain were found growing on moist soils which might even have been water-saturated for extended periods. Some stains at the heartwood boundary or transition zone were formed with neither bacteria nor fungi being detected in the discoloured wood. It was considered that the general site characteristics, together with soil pH and soil texture, may be involved. Similar strains have been observed in *Acer* and *Juglans* species (Good et al. 1955 in Hillis 1987;

Hart and McNabb 1963 in Hillis 1987; Scheffer and Cowling 1966 in Hillis 1987).

Panshin et al. (1964) stated that the highest resistance of wood is in transition between sapwood and hardwood. The inner heartwood of teak was less resistant to pathogen attack than the intermediate or outer heartwood (Kokutse et al. 2003). These similar results have been found in teak (Simatupang and Yamamoto 1999), as well as tropical species, e.g. *Piptadeniastrum africanum* (Deon et al. 1980), and temperate species, e.g. *Castanea sativa* (Dumoncaud 2001).

The function of this structure in heartwood formation is uncertain. The sharp boundaries have been associated with a sudden, and sometimes abrupt, aspiration of pits at the outer edge of the transition zone in conifers (and tyloses in some hardwoods) (Hillis 1987). It appeared that the presence of substantial lipid deposits in ray tissues of sapwood/heartwood zone could be a significant factor influencing the lateral impermeability of this region. This lipid could be redistributed during the flow of the preservative liquid into the wood leading to blockage of pits, or the lipid may exert a protective effect on pit membranes and prevent cracking during drying (Murphy et al. 1991).

Furthermore, Hillis (1987) stated that the consequent market decrease in permeability of these tissues results in their separation from the water stored in the inner sapwood. These changes at the outer periphery of the transition zones have been attributed to water tension in the inner sapwood at times of physiological drought in the rest of the tree so that gas accumulates in the inner layers, the pits aspirate, and tyloses form. Initiation of the formation of the transition zone in those species with regular heartwood formation is more likely to be caused by activation of the parenchyma in the inner sapwood than by water stress. As the parenchyma (and the resin canal epithelium when present) remain alive, the loss of water would take place from the surrounding tissues of the transition zone (Hillis 1987).

Although starch has been detected in the transition zone in some species, the translocation of a considerable amount of primary metabolites from the sapwood to the transition zones is required to form the high level of extractive found in some heartwoods. This could take place through the living ray parenchyma over a period of time, and the moisture required by those heartwoods wetter than the transition zone could pass through the same route (Hillis 1987).

In the majority of cases, the impermeable zone extended for most of the length of the sample (about 700 mm) (Hillis 1987; Murphy et al. 1991). This impermeability was not only seen on lateral faces but also affected the axial permeability/movement of preservative. As such, it may be a considerable barrier to the extent of penetration achievable in poplar round wood even in the case where sapwood and heartwood are both permeable. More work should be carried out before firm conclusions are drawn on the significance and causes of this effect (Murphy et al. 1991). Furthermore, Barnacle and Ampong (1974) stated that the

characteristics referred to the refractory intermediate zone (if common in all size of teak), could make difficult the

quality control of commercial treatment plant operations, viz, the determination of true retention figures.

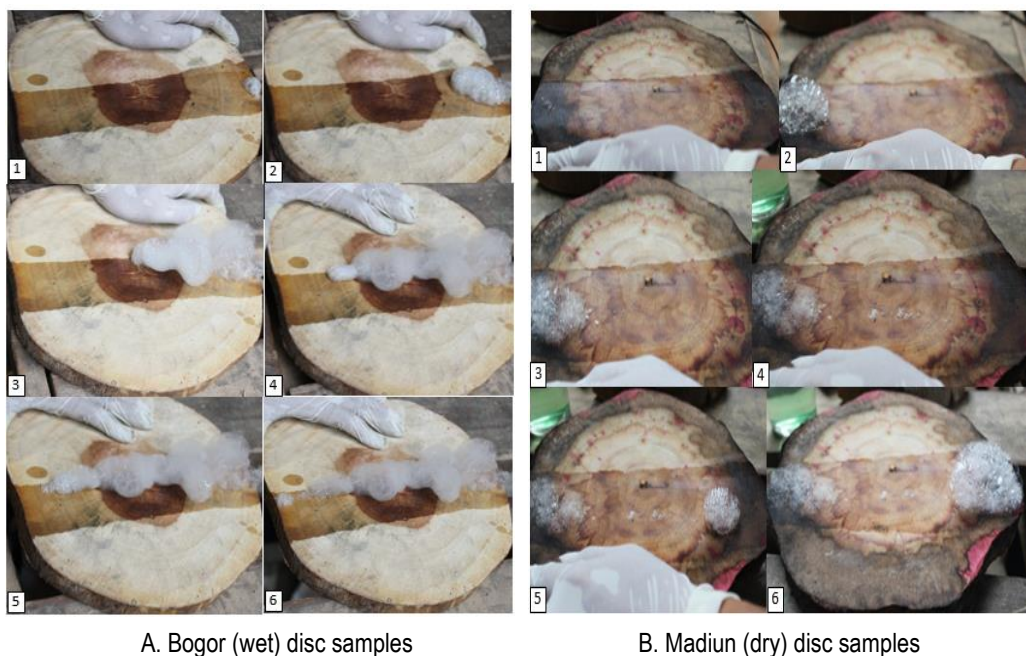


Figure 3. Bubble test results in the young-teak discs.

Figure 3A shows the bubbles appears in the upper surfaces (heartwood dan sapwood) of discs collected from Bogor (wet area). In contrast, bubbles do not appear in the heartwood part of discs originated from Madiun (dry area). The airflow from the bottom part of the discs creates bubble on the upper surfaces indicates the airflow is capable of moving the vapour through the wood. In the young-teak discs from Bogor area, the void volume is decreased caused by air pressure from the bottom part of the discs. It indicates the possible pressure treatment to enhance wood permeability. In young-teak discs collected from Madiun, the bubble only appears in the sapwood part. It indicates that the heartwood of young-teak discs originated from Madiun is less permeable and less possibility for possible pressure treatment. Possibly, it was because teak wood from dry site is thought to have more extractive materials than wood from wet locations (Damayanti *et al.* 2020).

### Conclusions

Red-dye water absorption and soap bubble methods are visible for wood permeability indicators. The heartwood of young-teak wood is less permeable than sapwood, and the transition zones are refractory. The transition zones may induce problem in preservative treatment, then additional pre-treatment should be conducted prior to preservative treatment. Bubble methods shows permeability indicators with pressurized air flows through the discs. The heartwood

and sapwood of young-teak discs collected from Bogor is permeable, while the heartwood of young-teak discs collected from Madiun is impermeable. The heartwood of young-teak originated from dry area is not readily treated with pressure preservative treatment.

### Acknowledgement

The first author would like to express her sincere gratitude and appreciation for the financial support provided by the Australian Centre for International Agricultural Research (ACIAR) during the PhD study at the University of Melbourne, Australia. Thanks are also directed to Unit Bagi Hasil Koperasi Perumahan Wanabhakti Nusantara (UBH-KPWN), Indonesian Ministry of Forestry, for providing the wood samples. All authors are equally contributed to the research and paper writing as main contributor.

### References

- Ahmad, S.A.; S.K. Chun. 2009. Observation of Liquid Permeability Related to Anatomical Characteristics in *Samanea saman*. Turkish Journal of Agriculture and Forestry 33(2): 155-163.
- Barnacle, J.E; F.F.K. Ampong. 1974. Refractory Intermediate Wood in Round Teak Fence Posts. Ghana Journal of Science 14 (2): 193-198.

- Butterfield, B.G.; B.A. Meylan. 1980. Three-dimensional Structure of Wood. An ultrastructure approach. 2<sup>nd</sup> Edition. Chapman and Hall, London.
- Damayanti, R.; B. Ozarska; J. Ilic; G. Pari; W. Dwianto; D.A. Indrawan; Krisdianto. 2020. Variation of Heartwood Proportion and Wood Colour from Fast Grown 5-Year-Old Teak. *Wood Research Journal* 11 (1): 1-11.
- Deon, G.; M. Chadenson; M. Hautville. 1980. Influence of Wood Natural Extractives on Its Decay Resistance. *Bois et Forets des Tropiques* 191: 75-90.
- Dumoncaud, O. 2001. Petits bois naturellement durables dans des usages extérieurs en vue de limiter l'emploi des traitements pas les pesticides: problèmes posés par l'utilisation du Chataignier (*Castanea sativa* Mill.) et intérêts d'un traitement de type friture à basse température. Thèse de Docteur de l'Engref.
- Falk, R.H. 2010. Wood as a Sustainable Building Material. In *Forest Products Society, Wood Handbook: Wood as an Engineering Material*. Forest Products Society, Madison, Wisconsin, USA.
- Hillis, W.E. 1987. Heartwood and Tree Exudates. Springer Series in Wood Science.
- Iskak, M. 2005. Produktivitas Tegakan Jati JPP Intensif sampai Umur 20 Tahun ke Depan. Pusat Pengembangan Sumber Daya Hutan, Perum Perhutani, Jakarta.
- Kamke, F.A.; J.N. Lee. 2007. Adhesive Penetration in Wood: A review. *Wood and Fiber Science* 39(2): 205-220.
- Kanninen, L.D.P.C.A.M. 2003. Heartwood, Sapwood, and Bark Content and Wood Dry Density of Young and Mature Teak (*Tectona grandis*) Trees Grown in Costa Rica. *Silva Fennica* 37: 45-54.
- Kokutse, A.D.; A. Stokes; H. Bailleres; C. Baudasse; K. Kokou. 2003. Heartwood and Natural Decay Resistance in Plantation Grown Teak (*Tectona grandis* L.f) from Togo, West Africa in Bhat (Eds): *Quality Timber Products of Teak from Sustainable Forest Management Proceeding*: 297-302.
- Krisdianto; G. Sumarni. 2006. Perbandingan persentase Volume Teras Kayu Jati Cepat Tumbuh dan Konvensional Umur 7 Tahun Asal Penajam, Kalimantan Timur. *Jurnal Penelitian Hasil Hutan* 24: 385-394.
- Kollman, F.P.; Cote, W.A.Jr. Cote,. 1968. Principles of Wood Science and Technology. Springer-Verlag Berlin Heidelberg.
- Lebow, S.T. 2010. Wood Preservation. In *Forest Products Society, Wood Handbook: Wood as an Engineering Material*. Forest Products Society, Madison, Wisconsin, USA.
- Murphy, R.J.; S.U Din; M.J. Stone. 1991. Observations on Preservative Penetration in Poplar. IRG 91-3662. Timber Technology Research Group, Dept. of Biology, Imperial College, London.
- Norton, J. 2012. Sub Activity 1.3.6 Chemical Treatment Boron/Vat Report. ACIAR FST 2006/117.
- Panshin, A.J.; C. de Zeeuw; H.P. Brown. 1964. Textbook of Wood Technology. Volume I: Structure, identification, uses, and properties of the commercial woods of the United States. McGraw-Hill Book Company. New York.
- Pokki, J.P.; V.V.; P. Laakso; Tikka; J. Aittamaa. 2010. Specific Permeability of Wood to Water Part 1: Longitudinal specific permeability of steamed, impregnated and kraft-cooked wood. *Industrial and Engineering Chemistry Research* 49(5): 2144-2154.
- Saranpaa, P. 2003. Wood Density and Growth. In Barnett, J.R. & Jeromidis, G. (Eds.) *Wood Quality and Its Biological Basis*. Garsington, UK: Backwell Publishing, Ltd.
- Simatupang, H.M.; K. Yamamoto. 1999. Properties of Teakwood (*Tectona grandis* L.f.) as Influenced by Wood Extractives and Its Importance for Tree Breeding. Regional Seminar on Site Technology and Productivity of Teak Plantations, Thailand.
- Soerianegara, I.; R. Lemmens. 1994. Plant Resources of South-East Asia 5 (1) Timber trees: Major commercial species. Leiden, Backhuys Publisher.
- Stamm, A.J. 1964. *Wood and Cellulose Science*. Ronald, New York.
- Yunianti, A.D. 2012. Nanostructure Characteristics of Cell Wall and their Relationship to Wood Quality (Case study in 7-year old cloned teak). PhD thesis. Bogor Agricultural University, Bogor.

Ratih Damayanti  
 Research Scientist - Anatomy and Quality of Ligno-cellulose Materials  
 Forest Products Research and Development Center,  
 FORDA, Ministry of Environment and Forestry,  
 Bogor, Indonesia  
 Tel. : +62 251- 8633378  
 Fax. : +62 251- 8633413  
 E-mail : ratih\_turmuzi@yahoo.com

Krisdianto  
 Wood Science Researcher  
 Forest Products Research and Development Center,  
 FORDA, Ministry of Environment and Forestry,  
 Bogor, Indonesia

Jugo Ilic  
 Associate Professor  
 Department of Forest and Ecosystem Sciences, The  
 University of Melbourne, Burnley Campus, Richmond,  
 Victoria, Australia

Gustan Pari  
Professor  
Forest Products Research and Development Center,  
FORDA, Ministry of Environment and Forestry,  
Bogor, Indonesia

Barbara Ozarska  
Professor  
Department of Forest and Ecosystem Sciences, The  
University of Melbourne, Burnley Campus, Richmond,  
Victoria, Australia

Peter Vinden  
Professor  
Department of Forest and Ecosystem Sciences, The  
University of Melbourne, Burnley Campus, Richmond,  
Victoria, Australia

# Improving Fire Resistance of Mahogany (*Swietenia macrophylla*) Wood Impregnated with Mixture of Borax and Boric Acid

Tomy Listyanto, Aditya Aji Pratama, Keisuke Ando, and Nobuaki Hattori

## Abstract

The study was aimed to investigate the effects of mixture of borax and boric acid concentration and different level of pressure on absorption, retention quantity, and fire resistance properties of mahogany (*Swietenia macrophylla*). A total of 50 samples of 9 x 19 x 1016 mm (ASTM E 69-02) were taken from mahogany lumber and then air-dried. A total of 45 samples were impregnated with aqueous solutions of borax and boric acid in three different concentrations (5, 7, and 9 %) and at three different pressure levels (5, 7.5, and 10 atm) for 2 hours pressure time, while 5 samples were lifted as control. Dried impregnated samples and untreated samples were feeding in flame with lid combustion method refers to ASTM E 69-02. Interestingly, the results showed that borax and boric acid effectively improved the fire resistance properties (mass losses, ignition temperature, maximum temperature, and smoke and smolder production) of mahogany wood. Mixtures of borax and boric acid at a concentration level of 9% and pressure of 10 atm are recommended to obtain the best results compare to the other interactions on combustion properties.

**Keywords:** Borax, boric acid, impregnation, mahogany-wood, fire resistance

## Introduction

Improving wood fire resistance has been an important aspect as wood, which is carbon-based cellulosic materials, tends to be combusted when subjected to heat at 270°C or with flammable materials at a lower temperature. Combustion of wooden materials is usually in the later stage of the fire but becomes more critical due to its proportion and position in building construction, especially in heavy timber construction. To improve safety, Inflammable wooden materials have been developed to reduce ignition and combustion properties. Fire retardant chemicals have been proposed and used to reduce the ignitability and combustibility of wood (Östman 2017). The pressure impregnation method has been widely suggested to increase adequate retention.

Boron components have been widely added as a booster to a fire-retardant compound to improve the effectiveness (Yu *et.al.* 2017a). Several studies have been conducted to investigate the effect of boron compounds on the fire resistance of wood. Recently, boron has been designated to be applied as a fire retardant for indoor wood furniture and other materials due to several advantages. Borax is one of the water-borne fire retardants which have several advantages such as against wood-destroying organism, reduce the flame spread, low eco-toxicity, and low volatility, colorless, odorless, and reasonable price (Winandy 1977; Yamaguchi 2001; Akbulut *et al.* 2004; Baysal and Yalinkilic 2005). However, borax has a disadvantage, which can promote glowing or smoldering. On the other hand, boric acid could reduce glowing and smoldering. There are no single chemicals normally used in fire retardants. Therefore boron-based buffers are important to be tested in fire-retardant treatments to significantly

reduce the severity of thermal degradation of commercial wood in Indonesia.

Mahogany (*Swietenia macrophylla*) is one of the highest commercially tropical timbers used, especially in Indonesia due to well-fast growth, specific gravity (0.61), and well machining properties (Martawijaya *et al.* 1989; Listyantp *et al.* 2016). However, it shows a lower durability class and resistant to fire. It is important to improve durability and fire resistance at the same time.

Wood impregnation process is influenced by several factors, both internal and external. Numerous important external factors are affecting the rate of impregnation such as the level of pressure, the composition and concentration of the impregnating solution, temperature, and, the time required for treatment. Impregnating solution contains boron components to mahogany wood are attracted to be studied. A proportion of borax and boric acid (1:1 w/w) with a concentration of 20% showing excellent results on fire-retardant properties on bamboo filament (Yu *et al.* 2017b). This study aimed to investigate the effect of interaction between three different concentrations (5, 7, and 9 %) and at three different pressure levels (5, 7.5, and 10 atm) for 2 hours pressure time on absorption, retention, mass losses, and combustion properties of mahogany wood. Observation of characteristic of thermal degradation is also essential as wood does not directly burn. Initial glowing point and flammable point of wood are observed concerning thermal conductivity and impregnated chemicals.

## Materials and Methods

### Materials

Research materials were mahogany lumber harvested at 25 years-old. A total of 50 samples of 9 x 19 x 1016 mm

(radial by tangential by longitudinal) (ASTM E 69-02) were taken from a mahogany board and then air-dried. Three groups concentration of borate, an aqueous solution of borax and boric acid mixed by weight (1.54/1; w/w) and dissolved in distillate water to a concentration 5%, 7%, and 9%, were prepared one day before treatment. For example, a volume of 30 liters aqueous borate 5% was made by mixing 1.5 liters borate and 28.5 liters distillate water.

### Method

The samples were impregnated with aqueous solutions of borax and boric acid in three different pressure levels; 5 atm, 7.5 atm, and 10 atm for 2 hours pressure time. Five replicates were used for each treatment variation. Five samples were lifted untreated as a control. The air-dried sample was observed on absorption (% w/w) and retention (% w/w). Samples were tested by feeding to the flames for four minutes with lid combustion method refers to

ASTM E 69-02. Analysis of variance and Tukey Honestly Significant Difference test at the 95% confidence level were conducted to perform statistical analysis.

## Results and Discussion

### Absorption and Retention

The absorption of treated samples was in the range of 197-356 kg/m<sup>3</sup> (Fig. 1). The retention was in the range between 9.85 kg/m<sup>3</sup> and 31.5 kg/m<sup>3</sup>. The results showed that no significant difference ( $p < 0.05$ ) was found in absorption and retention quantity. Pressure impregnation at 5, 7, and 9 atm, showed adequate effectiveness to increase retention quantity. Retention of borate solution is higher than that of prescribed in AWPA (2010) for timber foundation or structure in a hazardous condition. Therefore impregnating borate solution with a concentration of 5-9% with pressure level 5, 6, and 9 atm meet with the standard (AWPA 2010).

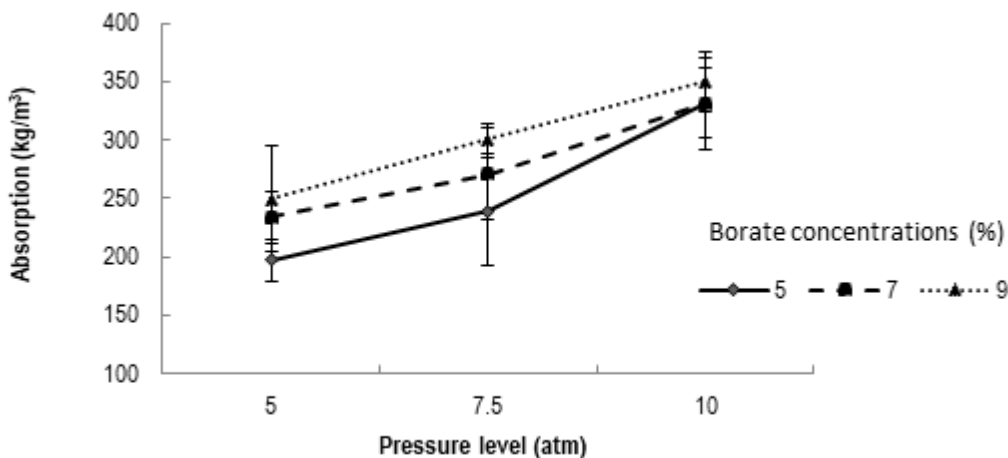


Figure 1. Graphic of Absorption (kg/m<sup>3</sup>) of treated samples in different concentration of borate (5, 7, and 9%) and different pressure (5, 7.5, and 10 atm).

### Mass Losses

Interestingly, during the fire resistance test, mass losses decreased significantly ( $p < 0.05$ ) in the treated samples (6.31%) compare to control (83%), as shown in Fig. 2. This result indicates that borax and boric acid are effective in increasing the fire resistance of mahogany lumber. This inline with the study of Awoyemi and Westermarck (2005) that boron-based buffers can be used in fire-retardant treatments and have been found to significantly reduce the severity of thermal degradation. In terms of physical mechanism, boron components effectively form a protective layer on the wood surface to avoid fire

(Kandola *et al.* 1996). In terms of chemical mechanism, boric acid seemed to successfully catalyze the dehydration and other oxygen-eliminating reactions of wood at a relatively low temperature, which contributes partly to the effects of boric acid for promoting charring and fire retardation of wood (Wang *et al.* 2004). However, there was no significant difference ( $p < 0.05$ ) in mass losses among treatments. Therefore, borate concentration 5% and pressure level 5 atm is sufficient to avoid mass losses during the fire retardant process on mahogany wood.

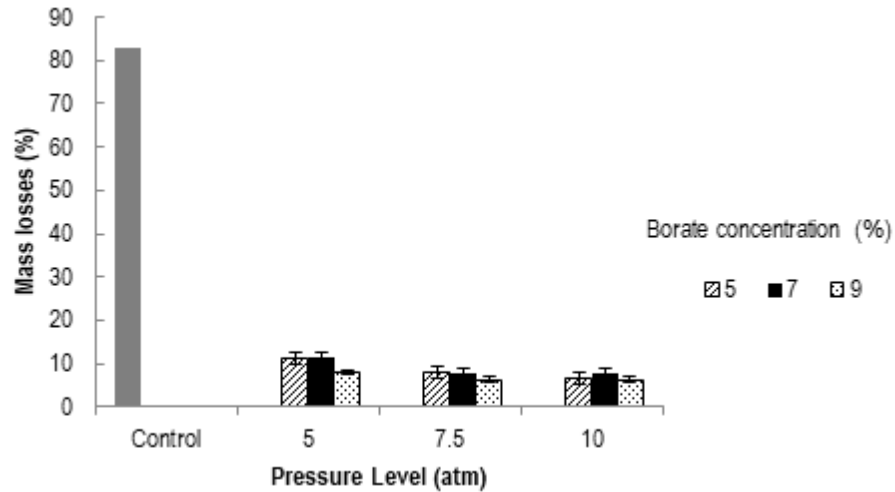


Figure 2. Graphic of mass losses (%) of treated samples in different concentration of borate (5, 7, and 9%) and different pressure (5, 7.5, and 10 atm), and control.

### Combustion Properties

Combustion properties of the treated sample improved significantly ( $p < 0.05$ ) compared to control. The initial ignition point of treated samples was at  $317^{\circ}\text{C}$  while the control sample in  $117^{\circ}\text{C}$  (Fig. 3). The ignition point of the untreated sample was very low which cause the lumber more was to be burned. The reason is that elements of boron could create a  $\text{B}_2\text{O}_3$  layer that spread across the surface of the wood sample to isolate oxygen and fire (Yang and Qing 2014). The result is consistent with the study by Yu *et al.*

(2017b). In addition, bound water is released by boron compound caused cooling material and absorbing the heat (Marosi *et al.* 2001). The highest initial smolder point of the treated sample was found in borate concentration 9% and pressures 10 atm ( $457^{\circ}\text{C}$ ). Besides, the maximum combustion temperature was also significantly ( $p < 0.05$ ) decreasing for the treated sample ( $573^{\circ}\text{C}$ ) compare to the control ( $653^{\circ}\text{C}$ ) as shown in Fig. 4.

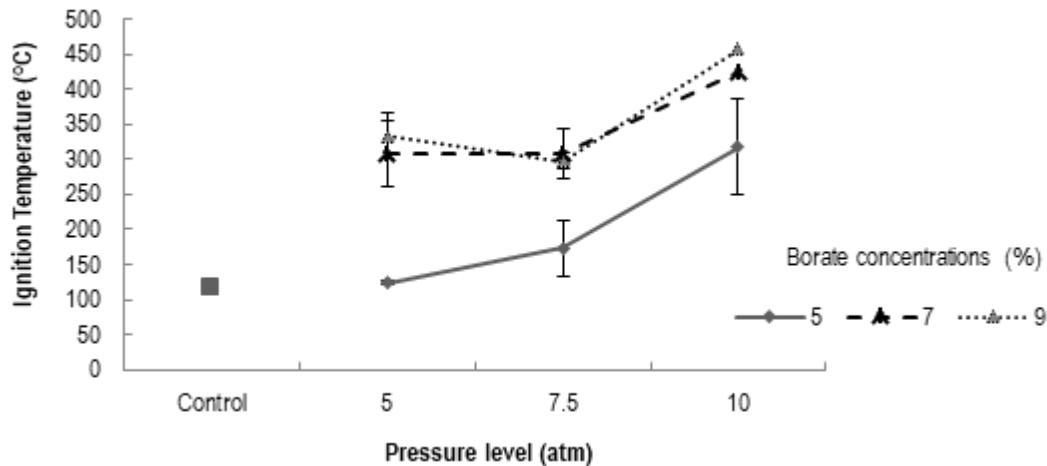


Figure 3. Graphic of Ignition temperature ( $^{\circ}\text{C}$ ) of treated samples in different concentration of borate (5, 7, and 9%) and different pressure (5, 7.5, and 10 atm), and control.

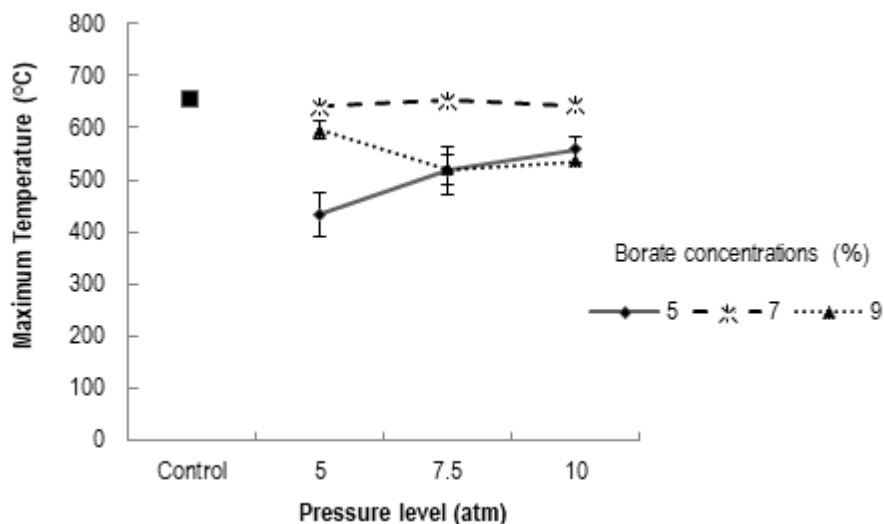


Figure 4. Graphic of maximum temperature (°C) of treated samples in different concentrations of borate (5, 7, and 9%) and different pressure (5, 7.5, and 10 atm), and control.

Visual inspection showed that the treated sample produce a slight smolder compare to the control (Table 1). Heavy smolder is an indicator that wood will be immediately burned. Boron reduces significantly smoke release and suppresses smolder. This result is corresponding to the result of research by Yu *et al.* (2017b) Therefore, the lower intensity of the smoke and smolder will be an important indicator to assess good fire retardant. Wang *et al.* (2004) proposed an explanation that mixtures of borax and boric acid could produce a char as a catalytic consequence at a lower temperature. A mixture of borax and boric acid (1:1) is an important factor in reducing smoke and smolder because borax has lower performance in suppressing smoke and smolder (Yu *et al.* 20017b)

In general, this study showed that impregnation with the combination of borax and boric acid in various concentrations and pressure showed effective improvement on fire resistance properties of mahogany. Impregnating boron components (borax and boric acid) with ratio mixing of 1:1 (w:w) at concentration level 9% and pressure 10 atm showed the best result compare to the other interactions on combustion properties. This corresponds to the study of Baysal *et al.* (2006), Yu *et al.* (2017b) that boric acid and borax had excellent fire-retardant effectiveness over untreated ones. This information is important because borax and boric acid could be considered wood preservative and fire retardant.

Table 1. Grade of based on visual observation

Concentration Levels [%]	Pressure [atm]	Smoke	Smolder
5%	5	medium	narrow
	7.5	medium	narrow
	10	medium	narrow
7%	5	narrow	narrow
	7.5	narrow	narrow
	10	medium	narrow
9%	5	narrow	almost none
	7.5	narrow	almost none
	10	narrow	almost none
Control		excessive	burned

## Conclusions

In summary, mixture of borax and boric acid in concentrations of 6, 7, and 9% and pressure level of 5, 7.5, 10 atm showed effective improvement on fire resistance properties (mass losses, ignition temperature, maximum temperature, and smoke and smolder production) of mahogany. Impregnation with mixture of borax and boric acid at concentration level 9% and pressure 10 atm showed the best result compare to the other interactions on combustion properties.

## References

- American Society for Testing and Materials. 2007. ASTM E 69-02: Standard Method for Combustible Properties of Treated Wood by the Fire-Tube Apparatus. ASTM International, West Conshohocken, United States.
- American Wood Preservers Association (AWPA). 2010. Annual Book of Standard. Grandbury, TX
- Akbulut, T.; S.N. Kartal; I.F. Green. 2004. Fibreboards Treated with N'-N-(1,8-Naphthyl) Hydroxylamine (NHA-Na), Borax, and Boric Acid. *Forest Products Journal* 54(10): 59-64.
- Awoyemi, L.; U. Westermarck. 2005. Effects of Borate Impregnation on the Response of Wood Strength to Heat Treatment. *Wood Science and Technology* 39 (6): 484-491.
- Baysal, E.; M.K. Yalinkilic. 2005. A Comparative Study on Stability and Decay Resistance of Some Environmentally Friendly Fire Retardant Boron Compound. *Wood Science and Technology* 39(3): 169-186.
- Baysal, E.; M. Altinok; M. Colak; S.K Ozaki; H. Toker. 2006. Fire Resistance of Douglas Fir (*Pseudotsuga menziesii*) Treated with Borates and Natural Extractives. *Bioresources Technology* 98(5): 1101-1105.
- Kandola, B.K.; A.R. Horrocks; D. Price; G.V. Coleman. 1996. Flame-Retardant Treatments of Cellulose and their Influence on the Mechanism of Cellulose Pyrolysis. *Journal of Macromolecular Science, Part C* 36(4): 721-794.
- Listyanto, T.; K. Ando; H. Yamauchi; N. Hattori. 2016. CO<sub>2</sub> Laser-Incised Teak and Mahogany Lumber Dried by Microwave and Steam Injection. *Forest Products Journal* 66(7-8): 461-466.
- Marosi, G.; A. Marton; P. Anna. 2002. Ceramic Precursor in Flame Retardant Systems. *Polymer Degradation & Stability* 77(2): 259-265.
- Martawijaya, A.; I. Kartasujana; Y.I Mandang; S.A. Prawira; K. Kadir. 1989. Atlas Kayu Indonesia Jilid I. Lembaga Penelitian dan Pengembangan. Ministry of Forestry. Indonesia. Bogor.
- Östman, B.A.L. 2017. Fire Performance of Wood Products and Timber Structures. *International Wood Products Journal* 8(2): 74-79.
- Wang, Q.; J. Li; J.E. Winandy. 2004. Chemical Mechanism of Fire Retardance of Boric Acid on Wood. *Wood Science and Technology* 38(5): 375-389.
- Winandy, J.E. 1977. Effects of Fire Retardant Retention, Borate Buffers, and Redrying Temperature after Treatment on Thermal-Induced Degradation. *Forest Products Journal* 47(6): 79-86.
- Yamaguchi, H. 2003. Silicic Acid: Boric Acid Complexes as Wood Preservatives: Ability of Treated Wood Resist to Termites and Combustion. *Wood Science and Technology* 37: 287-297.
- Yang, W.; Y. Qing. 2014. Effect Of Typical Boron Compounds on the Thermal Degradation and Combustion Properties of *Phyllostachys pubescent*. *Engineering Science* 16(4): 51-55.
- Yu, L.L.; F. Lu; D.C. Qin; H.Q. Ren; B.H. Fei. 2017a. Combustibility of Boron-Containing Fire Retardant Treated Bamboo Filament. *Wood and Fiber Science* 49(2): 125-133.
- Yu, L.; J. Cai; H. Li; F. Lu; D. Qin; B. Fei. 2017b. Effects of Boric Acid and/or Borax Treatments on the Fire Resistance of Bamboo Filament. *BioResources* 12(3): 5296-5307.

Tomy Listyanto and Aditya Aji Pratama  
Faculty of Forestry, Universitas Gadjah Mada  
Jl Agro No 1. Bulaksumur Yogyakarta  
E-mail: tomy.listyanto@gadjahmada.edu

Keisuke Ando and Nobuaki Hattori  
Tokyo University of Agriculture and Technology  
3-5-8 Saiwai cho, Fuchu shi, Tokyo, 183-8509, Japan.

# Strength and Stiffness Behavior of Earthquake Resistant Pedestrian LVL Timber Bridge

Bernardinus Herbudiman, Delima, and Yosafat Aji Pranata

## Abstract

A bridge is a structure which is used to connect two areas separate by obstacles. The environmental damage caused a number of reductions in the production of timber, and by that, the LVL timber which is a high quality processed or engineered timber is chosen. This research determined the design of the timber bridge structure for pedestrian with simple beam type and earthquake resistant. The load in this bridge is referring to the SNI 1725:2016 and SNI 2833:2008, the design of the girder and the connection is referring to SNI 7973:2013, and the deflection is referring to the LFRD for Highway Bridge Superstructures. The timber bridge is designed to have a span of 10 metres long and 3 metres wide. The modeling and designing of the wooden bridge are using an application called SAP2000 based on finite element analysis. Result obtained from this research indicated that the longitudinal dimension of the girder is 360 mm x 630 mm and the cross sectional dimension is 180 mm x 270 mm. The number of bolts and lock screws needed on the connection among the longitudinal girders are 40 pieces, between the longitudinal girders and the cross sectional girders is three pieces, and between the railing and the slab are two pieces. Based on the stiffness review, the results showed that the bridge deflection that occurred was lower than the permit deflection.

**Keywords:** Timber bridge, strength, stiffness, pedestrian bridge, LVL.

## Introduction

Government Regulation of the Republic of Indonesia Number 34 Year 2006 Article 86 Paragraph (3) explains that the definition of a bridge is a road that is located above water level and/or above ground level. Therefore, a bridge is a structure that functions to connect two areas with obstacles such as rivers or valleys. A wooden bridge is a type of bridge that uses wood materials with a fairly simple construction method when compared to other types of bridges. One type of bridge based on its use is a pedestrian bridge. Several pedestrian bridges or light vehicles are useful for connecting two areas that are separated by rivers, especially in tourism areas such as waterfall attractions, parks near rivers, and there tourist attractions (lakes).

LVL (Laminated Veneer Lumber) wood is processed wood consisting of a thin layer or wood veneers which are glued together on both sides and then pressurized. Gluing is carried out following the long direction of the wood. Therefore, the cross-sectional size of LVL wood can be adjusted so that it can be used as a building block for structural components. LVL wood is a potential material that can be used for the manufacture of wooden bridge structures.

Research related to the use of LVL Sengon (*Paraserianthes falcataria*) wood in building structures and bridges has been carried out, including for gable frame structures (Awaludin and Astuti 2016), for truss-type bridge structures for vehicles (Herbudiman *et al.* 2017), and as a component of shear wall structures in buildings (Awaludin *et al.* 2018). Research on the use of other wood such as coconut wood for a truss-type bridge structure has also

been carried out (Pranata *et al.* 2016), namely the truss-type bridge structure uses coconut wood.

This research determined the design of the timber bridge structure for pedestrian with simple beam type and earthquake resistant. The load in this bridge is referring to the SNI 1725:2016 (BSN 2016) and SNI 2833:2008 (BSN 2008), the design of the girder and the connection is referring to SNI 7973:2013 (BSN 2013), and the deflection is referring to the LFRD for Highway Bridge Superstructures. The timber bridge is designed to have a span of ten meters long and three meters wide. The modeling and designing of the wooden bridge are using an software called SAP2000 based on finite element analysis.

## Basic Theory

Wood is a material produced by nature so it is easier to obtain. Therefore, in ancient times wood material was used as an alternative choice for making infrastructure such as dwellings and bridges. However, along with increasingly advanced technology, wood materials are rarely used.

The simple beam bridge is the simplest bridge design among modern bridges today. Consists of horizontal road beams and is supported by beams that support the horizontal road. These stowed bearings withstand or resist the downward weight of the road body and the loads above it. This bridge model is more suitable for short distances such as connecting roads that are separated by rivers. In addition, if the road is getting longer, more beams must be systematically made to withstand the load so that it is not easily collapsed due to stress and compression. Simple beam bridges are usually intended for railways or connections between villages that are separated by rivers.

Wood is a natural resource which is currently used mostly in the plywood industry, such as furniture. It can be said that there is very little direct use of the bridge as the main construction. At least use wood as formwork for bridges.

A pedestrian bridge is a bridge whose bridge users are limited to pedestrians and light vehicles such as bicycles, motorbikes and light motorized vehicles with a maximum of three wheels can pass for emergencies. The dead load, live load, wind, and earthquake are used in pedestrian bridge modeling. Dead loads are all loads that come from the bridge's own weight, including all additional elements which are considered to be a permanent unit. Live loads are all loads that come from pedestrians, and are possible for light vehicles. Wind load is a load due to pressure moving horizontally. Meanwhile, earthquake loads are loads that work on a structure as a result of ground movement caused by an earthquake. Values for response spectrum curves can be obtained from the Research and Development Center of Settlement's website, these values vary depending on the area under review.

Based on SNI 1725: 2016 (BSN 2016) concerning Loading Standards for Bridges, the distribution of live loads for pedestrian loads and lane loads is pedestrian load, namely 5 KPa, wind load, namely the determined wind pressure is assumed to be caused by a planned wind with a basic speed ( $V_B$ ) of 90 to 126 km/hour. For bridges or bridge sections with an elevation higher than 10000 mm above the ground or water level, the design wind speed ( $V_{DZ}$ ) and the design wind pressure ( $P_D$ ) is calculated by the following equation:

$$V_{DZ} = 2,5V_0 \left( \frac{V_0}{V_B} \right) \ln \left( \frac{Z}{Z_0} \right) \quad (1)$$

$$P_D = P_B \left( \frac{V_{DZ}}{V_B} \right)^2 \quad (2)$$

with:

- $V_{DZ}$  = design wind speed, Z [km/hour],
- $V_{10}$  = wind speed at elevation 10000 mm [km/hour],
- $V_B$  = design wind speed which are 90 to 126 km/hour at elevation 1000 mm,
- Z = elevation of structure where wind load calculated ( $Z > 10000$  mm),
- $V_0$  = wind speed which is meteorology characteriztic [km/hour],
- $Z_0$  = length of friction at upstream bridge [mm],
- $P_B$  = basic wind pressure

Table 1.  $V_0$  and  $Z_0$  parameters for upstream condition (BSN 2016)

Parameter	Open field	Sub Urban	City
$V_0$ [km/hour]	13.2	17.6	19.3
$Z_0$ [mm]	70	1000	2500

Table 2. Basic wind pressure (BSN 2016)

Structural Component	Compression Wind [MPa]	Suction Wind [MPa]
Truss, column, and arch	0.0024	0,0012
Beam	0.0024	N/A
Flat surface	0.0019	N/A

The live load that is carried is 20 kN in the middle of the span. This can be seen in Table 1 in the *Surat Edaran* of the Minister of Public Works No. 02/SE/M/2010 concerning Implementation of Guidelines for Planning and Implementation of Suspension Bridge Construction for Pedestrians. Based on the Load and Resistance Factor Design (LRFD) for Highway Bridge Superstructures, the deflection requirements for wooden pedestrian bridges are determined using this regulation.

Based on the Design for Earthquake Loads according to the SNI 2833:2008 (BSN 2008), the planned earthquake load is calculated in association with the Peak Ground Accelerations (PGA) on the Indonesia earthquake hazard map. The calculation results and earthquake load curves can be obtained from secondary data (Pusperkim 2011).

## Methods

At the literature review stage, a study of basic theories related to the design of a simple earthquake-resistant wooden bridge for pedestrians was carried out. The sources of this literature study are books, literature, journals, articles and previous research. The data required for modeling after a literature study is wood mechanics data. The data is obtained based on primary and secondary data. Data on compressive strength parallel to fibers and shear strength of wood are obtained from the results of research conducted by Pranata *et al.* (2017). While the bending data obtained from laboratory test results. The data obtained refers to SNI, namely the timber flexural strength (SNI 03: 3959: 1995) and the timber shear strength (SNI 03: 3400: 1994).

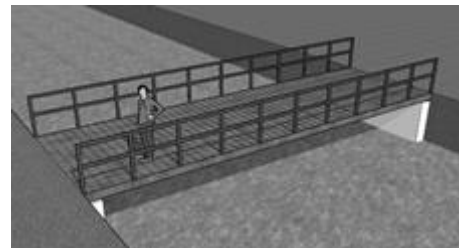


Figure 1. Schematic of Pedestrian bridge (Delima 2018).

This bridge is designed with a length of ten meters, a width of three meters, and a railing height of one meter. Bridge modeling using SAP2000 (CSI 2010) software can be done. This software is used because it has complete analysis features. Modeling carried out on SAP2000

software includes defining the wood mechanics data material, dimensions of girders and floor plates, and loading.

The aims of structural analysis are to determine the behavior of the bridge and the internal forces acting due to the working loads. Structural analysis can be done if the bridge modeling in SAP2000 software has been completed and is continued with run. Then the inner force will be obtained on the girder which is then used to design the dimensions of the girder and joints, as well as the deflection value to determine whether it meets the requirements or not. The design results are obtained when modeling, structural analysis, and connection design have been completed. The results of this design include the design of the bridge structure, girder dimensions, and the bridge connection system..

### Result and Discussion

After testing the flexural strength test in the laboratory, the flexural strength value is 36.175 MPa with an elastic modulus (E) value of 10.626 MPa obtained from Pranata *et al.* (2017) research.

Based on the results of the structural analysis (Delima 2018), the maximum deflection value was 19.888 mm, which means that it meets the requirements because it is greater than the permit deflection of 23.529 mm. The force in the greatest moment of the longitudinal girder occurs at frame 2, which is 129,806,282.7 Nmm. Meanwhile, the maximum shear force occurs at frame 3 which is 57,629.93 N. The force in the greatest moment of transverse girder occurs at frame 20, which is 23,721,459.45 Nmm. Meanwhile, the maximum shear force occurs at frame 22, which is 34,751.53 N. The diagram of the force in shear and moment can be seen respectively in Figure 2 and Figure 3.

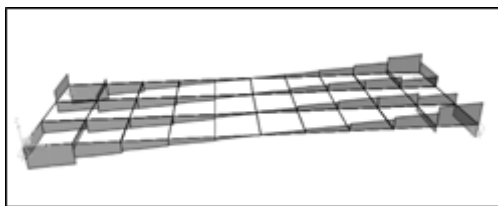


Figure 2. Shear load diagram (Delima 2018).

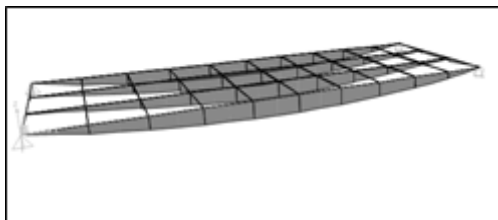


Figure 3. Flexural moment diagram (Delima 2018).

Based on the results of the SAP2000 analysis, the girder dimensions were checked based on the maximum moment and shear values. The size of LVL wood that can be ordered is a multiple of 90 mm. Therefore, dimensions of 360 x 630 mm for longitudinal girders and 180 x 270 mm for transverse girders are used. The top view of the plate less bridge can be seen in Figure 4, while the three-dimensional bridge can be seen in Figure 5.

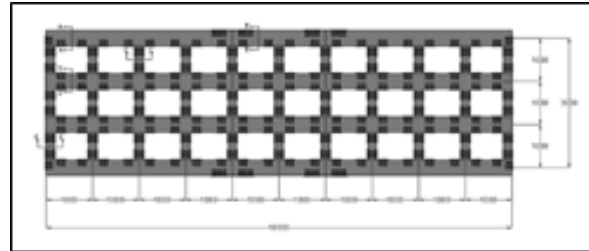


Figure 4. Top view of bridge (Delima 2018).



Figure 5. Schematic 3D of bridge (Delima 2018).

By using the dimensions of the girder, based on the results of analysis, the deflection value obtained was 19.888 mm. This value meets the requirements because it is less than the deflection value of the permit, namely 1/425 span or 23.529 mm.

Based on the value of the force in moment and shear of each point connected, the required number of bolts or lock screws is obtained. The joints are designed with a lock bolt or screw and a steel plate with a thickness of 10 mm. The bolts and lock screws used are 16 mm in diameter with a length of 15 cm for lock screws and 20 cm for bolts. For the connection between longitudinal girders, it takes 40 lock screws with a total of 5 bolts per row of 8 rows. As for the connection of the longitudinal girder and the transverse girder, 3 bolts are needed with a total of 3 bolts per row of 1 row. Details of the three-dimensional visible joints between the longitudinal girder and the longitudinal girder and the transverse girder can be seen in Figure 6 and Figure 7, respectively.

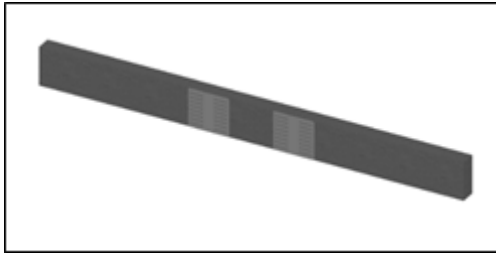


Figure 6. Connection of longitudinal girders (Delima 2018).

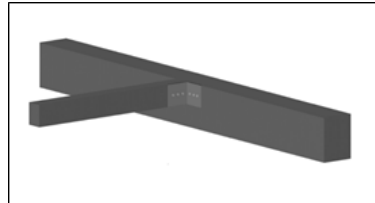


Figure 7. Connection of longitudinal girder and transverse beam (Delima 2018).

The design of girder dimensions and joint systems is planned with the following considerations:

1. The cross section of the T beam cannot be used because it cannot withstand the acting shear forces, so a square beam section is used.
2. The dimensions of the girder used are in accordance with the provisions, namely in multiples of 90 mm.
3. The connection between the longitudinal girders is connected between 3 girders, namely 4 m, 2 m, and 4 m, because the maximum length of the LVL log that can be used is 4 m.
4. The number of bolts and lock screws in the connection is planned with the largest internal force value, so that the number of bolts and lock screws for each type of connection is the same. This aims to improve efficiency in the field.

A lock screw with a diameter of 8 mm is used for the railing connection with the floor plate of the bridge, because if a larger diameter of the lock screw is used, the tip spacing and spacing requirements in the connection configuration will not be fulfilled, which will cause failure in the connection.

### Conclusions

After planning a simple earthquake-resistant wooden bridge for pedestrians for a span of 10 meters and a width of 3 meters, the dimensions of the girder used are 360 x 630 mm for the longitudinal girder and 180 x 270 mm for the transverse girder. By using these dimensions, the deflection that occurs has met the actual deflection requirements, which is 19.888 mm smaller than the 23.529 mm permit deflection. The connection is designed with a bolt and screw connection tool. Based on the planning results, the number of bolts and lock screws obtained is strong enough to withstand the loads acting on the bridge structure. The

diameter of the bolts and lock screws used is 16 mm. The connection between the longitudinal girder is designed with 40 locks screws, namely eight five-row locks screws, and the connection between the longitudinal girder and the transverse girder is designed with three bolts or lock screws in one row.

### Acknowledgement

The authors would like to thank Samko Timber and PT. Sumber Graha Sejahtera, for the support of the LVL wood material which was used as the raw material for the research material.

### References

- Awaludin, A.; P. Astuti. 2016. Study on Utilization of LVL Sengon (*Paraserianthes falcataria*) for Three-Hinged Gable Frame Structures, *International Journal of Engineering and Technology Innovation* 6 (3).
- Awaludin, A.; S. Shahidan; A. Basuki; S.S.M. Zuki; F.M. Nazri. 2018. Laminated Veneer Lumber (LVL) Sengon: An Innovative Sustainable Building Material In Indonesia. *International Journal of Integrated Engineering* 10(1);, 17-22.
- Badan Standardisasi Nasional (BSN). 2008. Perancangan jembatan terhadap beban gempa (SNI 2833:2008). Jakarta: Badan Standardisasi Nasional. (In Indonesian).
- Badan Standardisasi Nasional (BSN). 2013. Spesifikasi Desain untuk Konstruksi Kayu (SNI 7973:2013). Jakarta: Badan Standardisasi Nasional. (In Indonesian).
- Badan Standardisasi Nasional (BSN). 2016. Pembebanan Untuk Jembatan (SNI 1725:2016). Jakarta: Badan Standardisasi Nasional. (In Indonesian).
- Badan Standardisasi Nasional (BSN). 1995. SNI 03: 3959: 1995 Metode pengujian kuat lentur kayu di laboratorium (in Indonesian).
- Badan Standardisasi Nasional (BSN). 1994. SNI 03: 3400: 1994 Metode pengujian kuat geser kayu (in Indonesian).
- Computer and Structures, Inc. (CSI). 2010. SAP2000 user guide, Computer and Structures, Inc., 1995 University Ave. Berkeley, CA 94704, USA.
- Delima. 2018. Perencanaan Jembatan Kayu Tipe Balok Sederhana Tahan Gempa Untuk Pejalan Kaki, Tugas Akhir (tidak dipublikasikan). Program Studi Teknik Sipil, Fakultas Teknik Sipil Dan Perencanaan, Institut Teknologi Nasional Bandung. (In Indonesian).
- Government Regulation of the Republic of Indonesia Number 34 Year 2006 (Peraturan Pemerintah Republik Indonesia Nomor 34 Tahun 2006) Tentang Jalan. (In Indonesian).
- Herbudiman, B.; Y.A. Pranat; L. Pangest. 2017, Design of Pedestrian Truss Bridge with Sengon-Rubber Laminated Veneer Lumber, *The International*

- Conference on Eco Engineering Development 2017, IOP Publishing Conf. Series: Earth and Environmental Science Volume 109, doi: 10.1088/1755-1315/109/1/012029.
- Ministry of Public Works. 2010. Implementation of Guidelines for Planning and Implementation of Suspension Bridge Construction for Pedestrians, "Surat Edaran" No. 02/SE/M/2010 (in Indonesian).
- Pranata, Y.A.; A. Kevin. 2016, Struktur Jembatan Rangka Batang Kayu Kelapa, Seminar Nasional Pengabdian kepada Masyarakat, ISSN 2541-3805, Yogyakarta, Indonesia. (In Indonesian).
- Pranata, Y.A.; B. Herbudiman; N. Valiantine; L. Pangestu; F. Kawa. 2017. Experimental Report of Mechanical Properties of Sengon-Rubber LVL. Bandung: Universitas Kristen Maranatha Bandung (In Indonesian).
- Pusperkim. 2011. Desain Spektra Indonesia URL website: [http://puskim.pu.go.id/Aplikasi/desain\\_spektra\\_indonesia\\_2011](http://puskim.pu.go.id/Aplikasi/desain_spektra_indonesia_2011), Pusat Penelitian dan Pengembangan Pemukiman (In Indonesian).
- Pusat Penelitian dan Pengembangan Pemukiman. 2014. Retrieved from Teknologi Rumah Tahan Gempa dengan Struktur Kayu Laminasi (LVL): <http://puskim.pu.go.id/teknologi-rumah-tahan-gempa-dengan-struktur-kayu-laminasi-lvl> (In Indonesian).
- Bernardinus Herbudiman and Delima  
Department of Civil Engineering, Faculty of Civil Engineering and Planning, Institut Teknologi Nasional, Jl. PHH. Mustofa.23, Bandung 40124, West Java, Indonesia  
Tel : +62-8156145665  
E-mail : herbudimanb@yahoo.com, and delimanima@yahoo.com
- Yosafat Aji Pranata  
Department of Civil Engineering, Faculty of Engineering, Universitas Kristen Maranatha, Jl. Suria Sumantri 65, Bandung 40164, West Java, Indonesia  
Tel : +62-816623703  
E-mail : yosafat.ap@gmail.com or yosafat.ap@eng.maranatha.edu

# Reduction in Toxicity of Tectoquinone Against *Reticulitermes speratus* Kolbe Termites

Ganis Lukmandaru

## Abstract

This study investigated the cause of low toxicity against *Reticulitermes speratus* Kolbe termite species of teakwood (*Tectona grandis* L.f.) under natural conditions. Anti-termite test was conducted to evaluate the effectivity of four major components (tectoquinone, deoxylapachol, tecomaquinone, and squalene) of ethanol-benzene extracts in the teak heartwood. Tectoquinone was far superior to other components and exhibited both strong toxicity and antifeedancy. The strength reduction of tectoquinone bioactivity is assumed to be due to interaction with other major components. As squalene was found in considerable amounts or 1.8 to 13.1 times as high as the tectoquinone concentration in woods, termite feeding was set to the mixtures of tectoquinone and squalene in various ratios (1:1, 1:5, 1:10, and 1:20). It was revealed that squalene addition could decrease the termite mortality from 15% to 44% from its initial value (tectoquinone only). On the other hand, the mixtures only slightly reduced mass loss due to termite attacks.

**Keywords:** teak, tectoquinone, antifeedant, squalene, anti-termite.

## Introduction

Teak wood is valued for its high natural durability due to the presence of toxic components in the heartwood. Quinones and its derivatives are bioactive components against insects and fungi in teak heartwood (Haupt *et al.* 2003; Thulasidas *et al.* 2007; Niamké *et al.* 2011; 2013). Among them, in an isolated state, tectoquinone or 2-methylanthraquinone exhibits the highest level of toxicity against *Reticulitermes speratus* Kolbe termites (Lukmandaru and Ogiyama 2005; Lukmandaru 2012). Under natural conditions, however, teak heartwood only deters termites (Rudman *et al.* 1967; Lukmandaru and Takahashi, 2008; Lukmandaru 2011). Therefore, the exact causes of reduction in termiticidal properties should be explored.

Along with tectoquinone, several components i.e., squalene, desoxylapachol and its isomer, tectol, lapachol, 2-hydroxymethylanthraquinone, natural rubber or caoutchouc, sugar and sugar derivatives are found in varied amounts in teak extractives in which squalene shows significant concentrations (Yamamoto *et al.* 1998; Windeisen *et al.* 2003; Lukmandaru 2015). It is hypothesized that interaction between the primary compounds would affect the efficacy against termites. Thus, the main objectives of this work were to measure the content of main components in the heartwood, to evaluate the anti-termite activity of isolated compounds, as well as to test the toxicity levels of tectoquinone mixed with squalene.

## Material and Methods

Gas chromatography spectra and mass spectra were obtained using GC (Hitachi Model G-3 500) and GC-MS (JEOL XS mass spectrometry at 70 eV). The <sup>13</sup>C (in 400

MHz) and <sup>1</sup>H NMR (in 100 MHz) spectra were obtained using JEOL JNX-400 spectrometer.

## Sample Preparation and Extraction

Tectoquinone and squalene standard components were purchased from Kanto Chemical Co. (Japan). Tecomaquinone and deoxylapachol were isolated in a previous study (Lukmandaru 2013). In brief, teak heartwood meals (1 kg oven dried) were extracted by refluxing with *n*-hexane for 6 h. The obtained extract then was fractionated into neutral and acidic fractions by usual way. The neutral fraction (10 g) was then separated by column chromatography on a silica gel successively eluted with *n*-hexane, benzene, and ethyl acetate (EtOAc). Then, deoxylapachol was isolated by repeating column chromatography of the *n*-hexane fraction. By similar fashion, tecomaquinone was isolated from the EtOAc fraction. The GC-MS chromatogram of deoxylapachol was in agreement with that in the studies of Perry *et al.* (1991), and Lukmandaru and Takahashi (2009). Tecomaquinone was identified by comparing the NMR spectral data with previously published data (Lemos *et al.* 1999).

The outer heartwood samples from 8 individual trees were collected to study the component variation. The trees were planted in Perhutani Plantation, Randublatung, Central Java with a selected diameter range of 30-40 cm (class age IV). The wood meals were obtained by drilling from two opposite radii and were then combined to form a single sample. The wood meal (2 g) was subjected to Soxhlet extractions with ethanol-benzene (1:2, v/v) for 6 h. The extracts were dried on a rotary evaporator and the extractive contents were determined. The chemical structures of tested components are displayed in Figure 1.

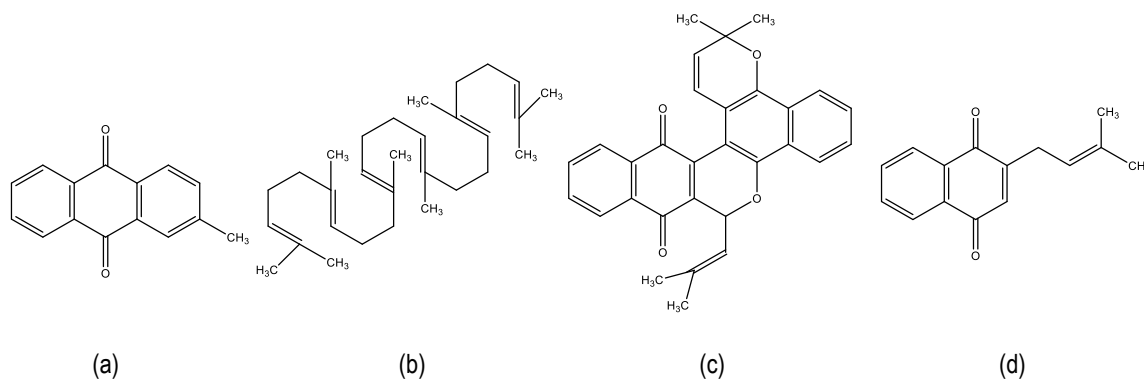


Figure 1. Chemical structures of tectoquinone (a), squalene (b), tecomaquinone (c), and deoxylapachol (d).

### Component Analysis

The extract (100 mg/mL) was injected to a GC/MS analysis. The setting conditions of column were: NB-1 bonded capillary 30 m, detector: FID, injection: splitless at 250°C. Carrier gas: He. Column temperature program: 120–300°C (programming 4°C min<sup>-1</sup>), detector and injector temperature at 250°C, held at 300°C for 15 min. MS conditions: temperature ionization voltage of 70 eV, transfer line at 250°C, acquisition mass ranging from 50–500 atomic mass unit. The content of the extractives was determined by comparison with using known amounts of reference samples and was expressed as percentages of oven-dried wood mass.

### Termites

*Reticulitermes speratus* termites (workers) were collected from a laboratory colony maintained in Yamagata University, Japan. The colonies were maintained on wood pieces in a controlled room for 3 months before the initiation of the test.

### Anti-termite Test

The no-choice test was conducted according to a method in a previous study (Lukmandaru and Ogiyama 2005). A test container was made from a Petri dish (diameter 9 cm, height 2 cm) containing 20 g moistened and sterilized sea sand in the bottom. Paper disc (diameter 8 mm; Whatmann International) were permeated with chloroform solution containing each of the test compounds (Fig. 2). The treatment retention of the single compound was 0.1%; 0.5 %, and 1.0% (w/w) per disc and 3 duplicates were applied for each sample. The retention of tectoquinone and squalene mixture was 2% whereas the ratios of tectoquinone and squalene were 1:1, 1:5, 1:10, and 1:20. The paper discs were dried at 60 °C for 2 h. In a control Petri dish, only chloroform was used. Fifty worker *Reticulitermes speratus* Kolbe termites were then added to each Petri dish. The petri dishes were covered in a dark chamber at 27°C and 80% relative humidity for the entire test period (10 days). The numbers of surviving and dead termites were counted. Finally, the discs were taken out, dried in the same manner, and the weight loss was weighted.



Figure 2. No-choice test against *Reticulitermes speratus* termites using paper discs.

## Statistical Analysis

The effect of different mixture concentration of components was analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test ( $p = 0.05$ ). The termite mortality rates (percentages) were transformed by the arcsine function for analysis. All statistical calculations were performed using SPSS-Win 16.0.

## Results and Discussion

### Component Variation

Ethanol-benzene mixture was used to extract most of the quinones in the teak heartwood. The extractive content ranged in 6-12%. The detected compounds by means GC/MS are displayed in the chromatogram (Figure 3). Some quinones (deoxylapachol and its isomer; tectoquinone, lapachol, tectol) as well as non-quinones (squalene, palmitic acid) were identified. The other 2 peaks were unknown compounds (Un1 and Un2). The compounds detected here were mostly similar to the earlier reports (Windeisen *et al.* 2003; Lukmandaru and Takahashi 2009; Lukmandaru 2015). The quantification of the extractives based on dry-wood showed squalene to be the most abundant component judging by the average values (Table 1) followed by

tectoquinone and tectol. On the other hand, palmitic acid and an unknown compound (Un2) were found in minor amounts. Although tectoquinone has been isolated by column chromatography, the green compound tectoquinone was not detected by GC-MS. This might be due to the column in the GC used in this experiment did not able to detect this compound. Unfortunately, the spot test by thin layer chromatography was not performed to confirm this finding.

It has been suggested that the bioactivity reduction of tectoquinone might be due to its interaction with other main compounds in the wood. Therefore, the amount of each compound was counted relative to tectoquinone content (Table 1). On the basis of 8 individual trees, it was found that the amount of tectoquinone was consistently higher than palmitic acid and UN 2 but lower compared to squalene (1.8 to 13.1 times). Compared to other quinones, the ratio values were varied depending on the individual samples. In term of natural durability against fungi, the ratio between tectoquinone and deoxylapachol could be a good indicator for predicting the resistance of teakwood against wood destroying fungi (Haupt *et al.* 2003). Tectol, unfortunately, was not isolated in this experiment. This dimeric naphthoquinone compound has never been mentioned in regards to teakwood durability (Windeisen *et al.* 2003).

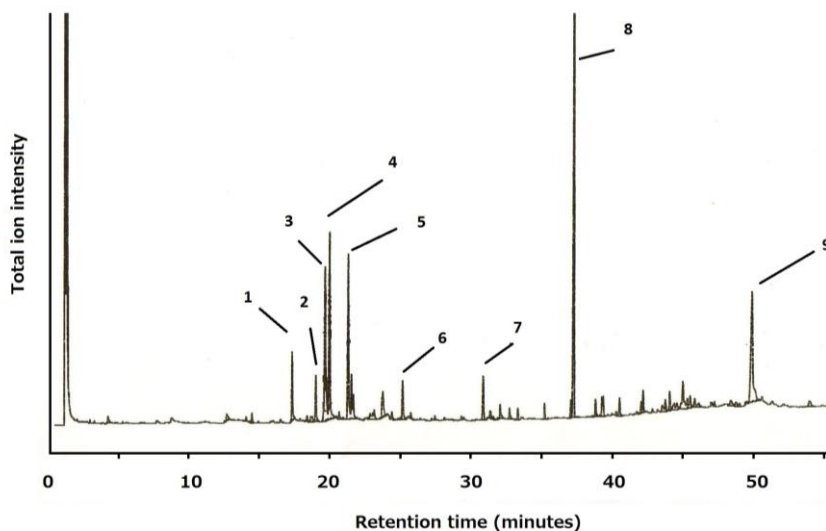


Figure 3. Gas chromatogram of ethanol-benzene extracts from teak heartwood. The detected components are peak 1 and 4 = deoxylapachol or its isomer; peak 2 = palmitic acid; peak 3 = lapachol; peak 5 = tectoquinone; peak 6 = unidentified compound 1; peak 7 = unidentified compound 2, peak 8 = squalene; and peak 9 = tectol.

Table 1. Tree characteristics and between-tree variation in the content of major compounds of ethanol-benzene extracts from teak heartwood expressed as percent of dry-wood meal weight.

Tree number	Dbh (cm)	EC (%)	DE(%)	PA (%)	LP(%)	ID (%)	TQ (%)	Un1 (%)	Un2 (%)	SQ (%)	TO (%)
1	39	6.44	0.06	0.01	0.03	0.22	0.12	0.12	0.08	1.52	0.23
2	33	9.57	0.06	0.05	0.14	0.21	0.59	0.10	0.09	1.13	0.55
3	35	9.25	0.35	0.01	0.73	0.75	0.34	0.42	0.06	1.34	1.00
4	37	7.95	0.15	0.06	0.09	0.34	0.10	0.10	0.05	0.49	0.27
5	38	7.02	0.36	0.07	0.06	0.20	0.08	0.40	0.02	0.96	0.33
6	30	11.32	0.15	0.70	0.11	0.46	0.84	0.19	0.00	1.54	0.95
7	32	8.58	0.98	0.13	0.48	0.67	0.31	0.33	0.12	1.79	1.01
8	35	11.72	0.05	0.01	0.03	0.05	0.18	0.00	0.02	0.52	0.15
Average		8.98	0.27	0.13	0.21	0.36	0.32	0.21	0.06	1.16	0.56

Remarks : Dbh : diameter breast height, EC = extractive content, DE = deoxylapachol, PA = palmitic acid, LP = lapachol, IDE = isodeoxylapachol, TQ = tectoquinone, Un1 = unknown 1, Un2 = unknown 2, SQ = squalene, TO = tectol

### Anti-termite Test of Single Components

In a literature review, tectoquinone and lapachol were effective antifeedant and toxic compounds against *Cryptotermes brevis* termites (Sandermann and Simatupang 1966). In another work, tectoquinone and deoxylapachol or its isomer were moderately correlated with the attacks of *Reticulitermes speratus* (Lukmandaru and Takahashi 2009). In this study, the anti-termite test was conducted on compounds where the quantity was similar to or higher than tectoquinone amounts, i.e., deoxylapachol, and squalene, in addition to tecomaquinone. The concentration interval was adjusted by its range in the natural condition (Table 1). The results of three concentrations (0.1%, 0.5%, and 1.0%, based on paper disc weight) are presented in Figure 3.

In general, the higher concentration caused a lower mass loss and higher mortality rate. As expected, tectoquinone showed the highest toxicity effect (mortality rates: 33-98%) and lowest mass loss (0.79-1.79 mg).

Beside tectoquinone, no other compounds showed a strong termiticidal activity. However, moderate levels of antifeedant activity or feeding deterrent effect) were exhibited by deoxylapachol, squalene, and tecomaquinone, particularly for the concentration of 1.0%. It was indicated by mass loss rate (control mass loss = 6.19 mg as the values were 3.27 mg; 3.92 mg, and 4.28 mg, respectively). For comparison, however, tectoquinone was not shown to be toxic and was only deterred *Nasutitermes exitiosus* (Rudman and Gay 1961). This condition probably was caused by a differential feeding preference among termite species. In an earlier report (Lukmandaru and Ogiyama 2005), tecomaquinone in high dosage (5 %, w/w) exhibited a strong antifeedant activity (mass loss of 0.04 mg) against the same species (*Reticulitermes speratus*) but in a low level of termiticidal activity. Thus, this result confirms the effectivity of high concentrations as a deterrent.

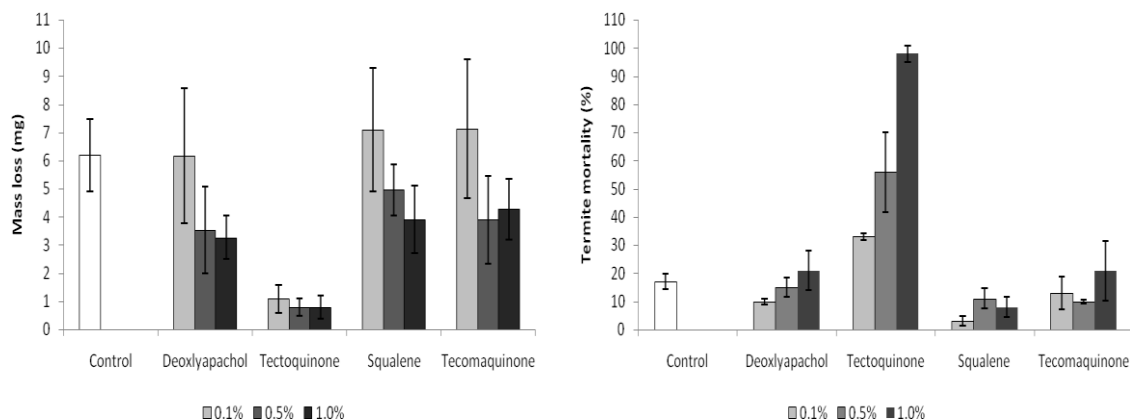


Figure 4. Mass loss (a) and mortality rate (b) against *Reticulitermes speratus* on 10-day observation of teakwood components by treatment retention (based on paper disc weight). Mean of 3 replications (with the standard deviation error bar).

### Anti-termite Test for Tectoquinone-squalene Mixtures

As tectoquinone exhibited the highest termite mortality rate, the interaction with other compounds warrants future study. In this experiment, squalene, which had a concentration that was consistently higher than tectoquinone in the wood (Table 1), was mixed with tectoquinone. It is assumed that squalene in a high amount would reduce the effectivity of tectoquinone. The total concentration of mixture was determined for 2% of mass weight in different ratios i.e. 1:1, 5:1, 10:1, and 20:1 or equivalent to tectoquinone concentration of 1.0%, 0.4%, 0.2%, and 0.1%, respectively. The ratios range was set to variation found in Table 1 and the results are summarized in Figure 4.

The ANOVA revealed that there was a statistically significant difference for component factor ( $p < 0.01$ ) for both mass loss and termite mortality levels. By Duncan's test, it was found that the mass loss was less affected (mass loss = 1.2 mg) even until squalene concentration was 20 times as high as tectoquinone concentration and compared to tectoquinone alone in 1% concentration (mass loss = 1.09 mg). This finding suggests that squalene partly affects the antifeedancy against termites. On the other hand, the 1:5 ratio had a mass loss of 2.69 mg. Furthermore it was also found that the addition of squalene in the mixture 1:1 did not follow the trend of mass loss in a linear pattern. This mixture proved to more effective than tectoquinone alone. This was an unexpected result as different ratios gave lower mass loss values. The cause of this result as yet unknown, but may be related to the effectivity of squalene to dissolve tectoquinone in the mixture or distribution of tectoquinone in the paper disc.

With regard to toxicity, tectoquinone alone (in 1% concentration) significantly showed the highest value (mortality rate = 85%). After mixing tectoquinone with squalene, a decrease in termite mortality was observed although not linearly related. The average values for squalene/tectoquinone ratio of 1:1, 5:1, 10:1, and 20:1 were 72%, 65%, 49% and 47.5%, respectively. The highest termite mortality reduction was equal to 44% which showed by 20:1 ratio. Furthermore no significant difference was noted in Duncan's test between termite mortality level of 20:1 and 10:1 ratios. The ratio of 1:1 or equal to 1% tectoquinone and 1% squalene concentrations showed termite mortality of 72% or 15% reduction from the initial value.

The high content of squalene is theorized to affect the natural durability of teakwood in the form of a hydrophobic barrier (Windeisen *et al.* 2003). In this experiment, it was demonstrated that squalene could reduce termite mortality considerably but less for feeding deterrent. However, other components in a significant amount that could not be detected by GC-MS may also contribute towards anti-termite properties. Previously, it was postulated that natural rubber or caoutchouc in teak might improve the decay resistance by a synergetic effect with bioactive extracts (Yamamoto *et al.* 1998). The other components might be non-structural sugars that have been associated with decay resistance in teak (Niamké *et al.* 2011). Therefore, further research is needed to explore the role of other the other inactive components in teak and their ability to affect the natural durability.

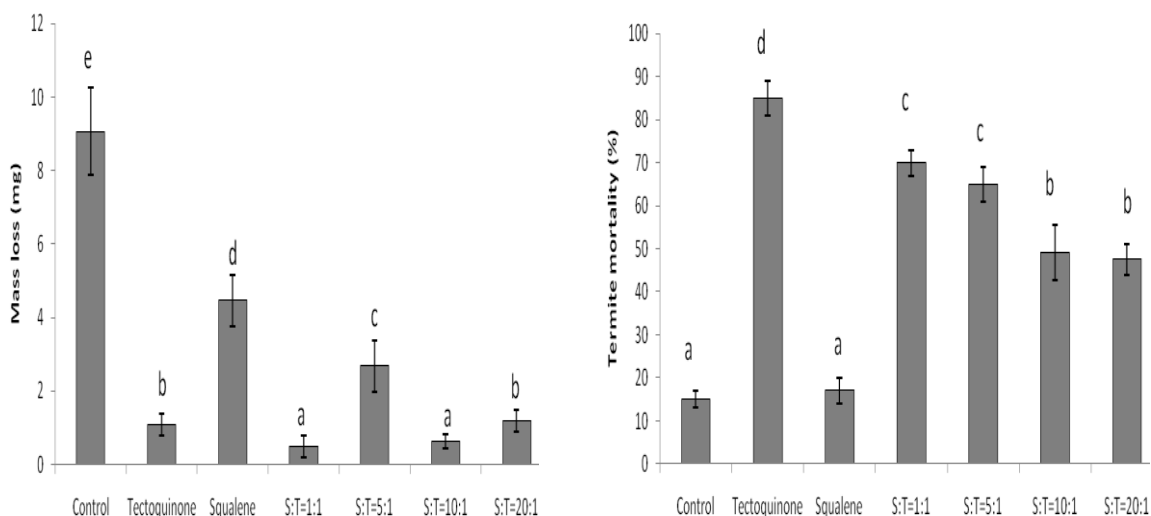


Figure 5. Mass loss (a) and mortality rate (b) against *Reticulitermes speratus* on 10-day observation of squalene and tectoquinone mixtures. Treatment retention (based on paper disc weight) of only squalene or tectoquinone was 1%. Treatment retention of squalene and tectoquinone mixtures was 2%. Mean of 3 replications (with the standard deviation error bar). The same letters on the same graphic are not statistically different at  $p < 0.05$  by Duncan's test.

## Conclusions

Under natural condition, teak wood is not toxic but merely deters the *Reticulitermes speratus* Kolbe termites. Therefore, the anti-termite activity of main extractive components of teak (tectoquinone, tecomaquinone, deoxylapachol, and squalene) was evaluated by no-choice tests. The activities of these compounds were evaluated in several concentrations (0.1%, 0.5%, and 1.0%, based on paper disc weight). It was revealed that only tectoquinone showed strong toxicity whereas the other compounds merely showed a moderate level of deterrent effects or antifeedant properties. As the most abundant constituent in the ethanol-benzene extracts of teakwood, the effect of squalene to tectoquinone efficacy was also evaluated. Various mixture concentrations have been set in the proportions present in teak (squalene/tectoquinone = 1:1, 1:5, 1:10, and :20). It was observed that squalene reduced the termite mortality of tectoquinone 15% to 44% from its initial value (single tectoquinone compound). The mass loss that indicated the repellency, however, was less affected by squalene addition.

## Acknowledgements

The author is grateful to Prof. Koetsu Takahashi (Faculty of Agriculture, Yamagata University, Japan) for facilitating this research. The author is also thankful to Mr. Untoro Tri Kurniawan (Perhutani Enterprise) for providing the samples.

## References

- Haupt, M.; H. Leithoff; D. Meier; J. Puls; H.G. Richter; O. Faix. 2003. Heartwood extractives and natural durability of plantation-grown teakwood (*Tectona grandis* L.)—a case study. *Holz als Roh-und Werkstoff* 61: 473-474.
- Lemos, T.G.; S.M. Costa; O.L. Pessoa; R. Braz-Filho. 1999. Total assignment of <sup>1</sup>H and <sup>13</sup>C NMR spectra of tectol and tecomaquinone. *Magnetic Resonance of Chemistry* 37: 908-911.
- Lukmandaru, G. 2011. Variability in the natural termite resistance of plantation teak wood and its relations with wood extractive content and color properties. *Journal of Forestry Research* 8(1): 17-31.
- Lukmandaru, G. 2012. Bioactive extracts from neutrals of teakwood (*Tectona grandis* L.f.). Proceedings of the 3<sup>rd</sup> International Symposium of Indonesian Wood Research Society. Yogyakarta, p. 328-332.
- Lukmandaru, G. 2013. Antifungal activities of certain components of teak wood extractives. *Jurnal Ilmu dan Teknologi Kayu Tropis* 11(1) : 11-18.
- Lukmandaru, G. 2015. Quinones distribution of teak wood grown in community forest. *Jurnal Ilmu dan Teknologi Kayu Tropis* 13(2): 193-204.
- Lukmandaru, G.; K. Takahashi. 2008. Variation in the natural termite resistance of teak (*Tectona grandis* Linn fil.) wood as a function of tree age. *Annals of Forest Science* 65(5): 708 p1-p8.
- Lukmandaru, G.; K. Takahashi. 2009. Radial distribution of quinones in plantation teak (*Tectona grandis* L.f.). *Annals of Forest Science* 66(6): 605 p1 – p9
- Lukmandaru, G.; K. Ogiyama. 2005. Bioactive compounds from ethyl acetate extract of teakwood (*Tectona grandis* L.f.). Proceedings of the 6<sup>th</sup> International Wood Science Symposium LIPI-JSPS Core, Bali. pp. 346-350.
- Niamké, F.B.; N. Amusant; J.P. Charpentier; G. Chaix; Y. Baissac; N. Boutahar; A.A. Adima; S.K. Coulibaly; C.J. Allemand. 2011. Relationships between biochemical attributes (non-structural carbohydrates and phenolics) and natural durability against fungi in dry teak wood (*Tectona grandis* L. f.). *Annals of Forest Science* 68: 201–211.
- Perry, N.B., J.W. Blunt; M.H.G. Munro. 1991. A cytotoxic and antifungal 1,4 naphthaquinone and related compounds from a New Zealand brown alga, *Landsburgia quercifolia*. *Journal of Natural Product* 54: 978-985.
- Rudman, P.; F.J. Gay. 1961. The causes natural durability in timber part VI. Measurement of anti-termite properties of anthraquinones from *Tectona grandis* L.f. by rapid semi-micro method. *Holzforschung* 15: 117-120
- Rudman, P.; E.W.B. Da Costa; F.J. Gay 1967. Wood quality in plus trees of teak (*Tectona grandis* L. f.) : an assessment of decay and termite resistance. *Sylvae Geneticae* 16: 102 -105.
- Sandermann, W.; M.H. Simatupang. 1966. On the chemistry and biochemistry of teakwood (*Tectona grandis* L. fil). *Holz als Roh- und Werkstoff* 24: 190–204.
- Thulasidas, P.K.; K.M. Bhat. 2007. Chemical extractive compounds determining the brown-rot decay resistance of teak wood. *Holz als Roh-und Werkstoff* 65: 121-124.
- Windeisen, E.; A. Klassen; G. Wegener. 2003. On the chemical characterization of plantation teakwood (*Tectona grandis* L.) from Panama. *Holz als Roh-und Werkstoff* 61:416-418.
- Yamamoto, K.; M.H. Simatupang; R. Hashim. 1998. Caoutchouc in teak wood (*Tectona grandis* L.f): formation, location, influence on sunlight irradiation, hydrophobicity and decay resistance. *Holz als Roh-und Werkstoff* 56: 201-209.

Ganis Lukmandaru  
Department of Forest Products Technology, Faculty of  
Forestry, Universitas Gadjah Mada,  
Jl. Agro No.1, Bulaksumur, Yogyakarta 55281, Indonesia

Tel. : +6274 550541  
Fax. : +6274 550541  
Email : [glukmandaru@ugm.ac.id](mailto:glukmandaru@ugm.ac.id)

# Antifungal Activity of Triterpenoids and Steroids Isolated from *Pinus merkusii* Bark Against *Phanerochaete chrysosporium*

Masendra, Brandon Aristo Verick Purba, and Ganis Lukmandaru

## Abstract

The outer part of a tree, known as the bark, protects the internal tissues from external conditions and attacks from microorganisms. Its antifungal activities are due to the presence of chemicals in this bark. This study aimed to evaluating the toxicity of triterpenoids and steroids from the bark of *Pinus merkusii* against *Phanerochaete chrysosporium* (white-rot). The triterpenoids and steroids were isolated from *n*-hexane extract of the bark through column chromatography. Then, the antifungal activity was evaluated by measuring the growth rate of the fungus on potato dextrose agar (PDA) medium in a Petri dish. The isolation resulted in three triterpenoids (3 $\beta$ -methoxyserrate-14-en-21-one, serrate-14-en-3 $\beta$ ,21 $\beta$ -diol, 3 $\alpha$ ,21 $\beta$ -dimethoxy- $\Delta$ 14-serratene) and two steroids ( $\beta$ -sitosterol, Stigmast-4-en-3-one). There was more of antifungal activity for  $\beta$ -sitosterol and serrate-14-en-3 $\beta$ ,21 $\beta$ -diol, due to presence of hydroxyl bond and their hydrophobicity.

**Keywords:** *Pinus merkusii*, bark, steroids, triterpenoids, antifungal, white-rot

## Introduction

Bark is an important part, covering almost the whole part of a tree. According to Wittstock and Gershenzon (2002); Alfredsen *et al.* (2008); and Pásztor *et al.* (2016), it protects the inner part of the tree from extreme and other external conditions such fire, cold, herbivore, pathogens, as well as detrimental insects and fungi. One of the ways it defends the tree is by storing secondary metabolites toxic to its enemies. Previous researches showed that its phenolics and lipophilic components possess bioactivity actions against various pests, disease-causing organisms, and wood decaying fungi such as white and brown-rot fungi (Wijayanto *et al.* 2015; Shreaz *et al.* 2016; Lomeli-Ramírez *et al.* 2016; Özgenç *et al.* 2017).

*Pinus merkusii* Jungh & de Vriese is a conifer of Pinaceae family, usually used as raw material in the production of pulp and paper, gum rosin as well as turpentine in Indonesia. Similarly, previous studies on *P. merkusii* bark showed the presence of phenolic and lipophilic extractives, also in its knots and stem wood (Wijayanto *et al.* 2015; Masendra *et al.* 2018a; Masendra *et al.* 2019; Masendra *et al.* 2020). In addition, Wijayanto *et al.* (2015) reported that the phenolic stilbenes of *P. merkusii* wood showed antifungal activity against *Trametes versicolor* (white-rot) and *Poria placenta* (brown-rot). Additionally, triterpenoids and steroids from *Astilbe myriantha* roots, *Ficus drupacea* bark and *Pallavicinia lyellii* have been reported to exhibit antifungal activity (Subhisha and Subramoniam 2005; Song *et al.* 2011; Yessoufou *et al.* 2015). Furthermore, a previous work by Masendra *et al.* (2018b) showed the isolation of three triterpenoids and two steroids from the bark of *P. merkusii*. Based on this

background, the aim of this study was to evaluate the toxicity of triterpenoids and steroids isolated from the bark of *P. merkusii* against *P. chrysosporium*. The use of the *P. chrysosporium* in this study due to the capability of this white-rot basidiomycete fungi to degrade lignin and also was used to test five species of woods in Brazil as their natural resistance (Oliveira *et al.* 2010).

## Materials and Methods

### Extraction and Isolation

Silica gel (60 N, spherical 63  $\mu$ m to 210  $\mu$ m; neutral Kanto Chemical Co., Inc., Tokyo, Japan) was applied for column chromatography with a glass column (40  $\times$  2.5 cm inner diameter). Aluminum sheets pre-coated with silica gel 60 F254 (Merck, Kenilworth, NJ, USA) were used for thin-layer chromatography (TLC). The spots were visualized using ultraviolet (UV) light irradiation ( $\lambda$  = 254 and 360 nm) by spraying with vaniline-sulfuric acid, for color testing, followed by heating at 150  $^{\circ}$ C for 10 min.

The *P. merkusii* bark was subjected to extraction with *n*-hexane for two weeks under room temperature and then evaporated until air dry before weighing. The *n*-hexane extract with a yellow color was 1.59 g but after separation, 1.0 g was chromatographed into Silicon gel column chromatography (SiGCC). This resulted in 63.1 mg  $\beta$ -sitosterol, 13.9 mg Stigmast-4-en-3-one, 14.8 mg 3 $\beta$ -methoxyserrate-14-en-21-one, 71.0 mg serrate-14-en-3 $\beta$ ,21 $\beta$ -diol, and 1.0 mg 3 $\alpha$ ,21 $\beta$ -dimethoxy- $\Delta$ 14-serratene. The chemical structures of isolated compounds are shown in Figure 1.

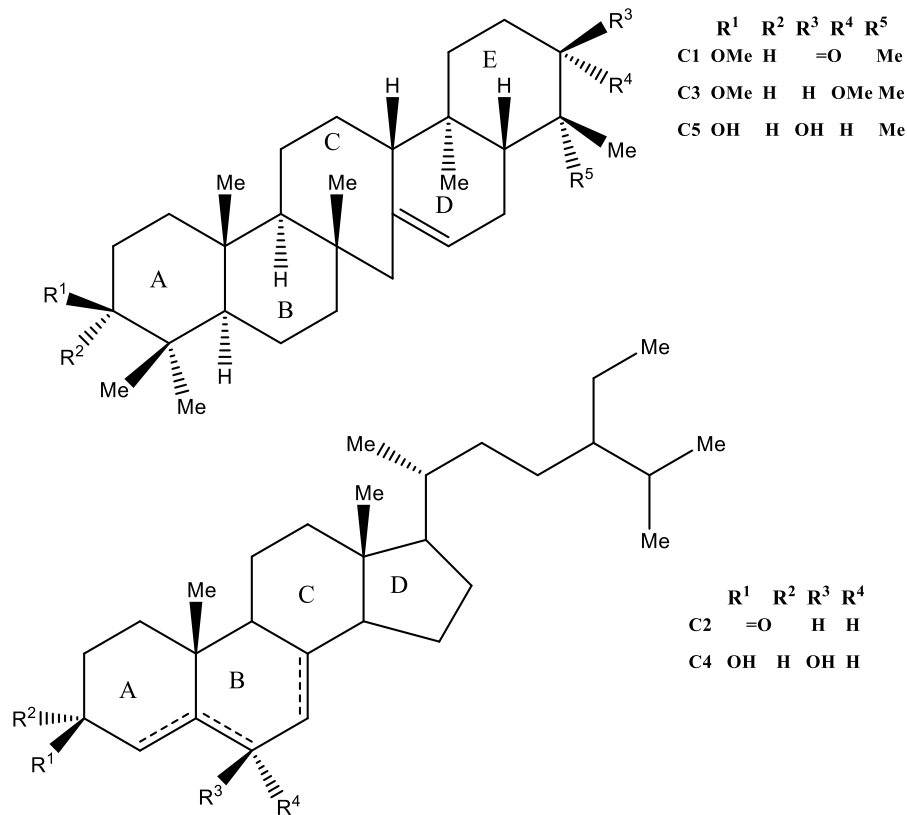


Figure 1. Chemical structure of isolated compounds from *P. merkusii* bark; **C1**: 3 $\beta$ - methoxyserrat-14-en-21-one, **C2**: stigmast-4-en-3-one, **C3**: 3 $\alpha$ ,21 $\beta$ - dimethoxy- $\Delta$ 14-serratene, **C4**:  $\beta$ -sitosterol, and **C5**: Serrate-14-en-3 $\beta$ ,21 $\beta$ -diol.

**3 $\beta$ -Methoxyserrat-14-en-21-one (C1)**. EI-MS  $m/z$  454 ( $M^+$ ;  $C_{31}H_{50}O_2$ , 43), 221 (100), 218 (73), and 135 (63). The  $^1H$ -NMR ( $CDCl_3$ ):  $\delta$  0.93 (3H, s, H-23-Me), 0.73 (3H, s, H-24-Me), 0.77 (3H, s, H-25-Me), 0.80 (3H, s, H-26-Me), 0.90 (3H, s, H-28-Me), 1.02 (3H, s, H-29-Me), 1.06 (3H, s, H-30-Me), 2.60 (1H, dd,  $J = 11.7$  and  $4.1$  Hz, H-3 $\alpha$ ), 2.73 (1H, dt,  $J = 14.8$  and  $5.5$  Hz, H-20), 3.33 (3H, s, H-3-OMe), and 5.35 (1H, brs, H-15).  $^{13}C$ -NMR ( $CDCl_3$ ):  $\delta$  38.5 (C-1), 22.3 (C-2), 88.4 (C-3), 38.2 (C-4), 56.2 (C-5), 18.7 (C-6), 45.1 (C-7), 38.9 (C-8), 56.2 (C-9), 36.1 (C-10), 25.5 (C-11), 27.2 (C-12), 62.7 (C-13), 138.3 (C-14), 122 (C-15), 24.4 (C-16), 51.2 (C-17), 37.1 (C-18), 38.3 (C-19), 34.8 (C-20), 217.1 (C-21), 47.6 (C-22), 15.7 (C-23), 19.8 (C-24), 28.1 (C-25), 16.2 (C-26), 55.9 (C-27), 12.9 (C-28), 24.5 (C-29), 21.6 (C-30) 57.5 (OMe).

**Stigmast-4-en-3-one (C2)**. The EI-MS  $m/z$  412 ( $M^+$ ;  $C_{29}H_{48}O$ , 39), 397 (8), 370 (14), 289 (22), 229 (38), and 124 (100). The  $^1H$ -NMR ( $CDCl_3$ ) results were as follows:  $\delta$  1.49, 1.24 (each 1 H, m, H-1), 2.36, 2.25 (each 1 H, m, H-2), 5.70 (1H, br, s, H-4), 2.02, 1.90 (each 1H, m, H-6), 1.18, 1.42 (each 1 H, m, H-7), 1.41 (1H, m, H-8, H-9), 1.56, 1.27 (each 1H, m, H-11), 1.57, 1.31 (each 1 H, m, H-12), 1.02 (1H, m, H-14), 1.62, 1.35 (each 1 H, m, H-15, H-16), 1.13 (1H, m, H-17), 0.69 (3H, s, H-18), 1.16 (3H, s, H-19), 1.65 (1H, s, H-20), 0.84 (3H, d  $J = 6.9$  Hz, H-21), 1.27, 1.27 (each 1 H, m,

H-22, H-23), 1.48 (1H, m, H-24), 1.83 (1H, m, H-25), 0.80 (3H, d,  $J = 6.9$  Hz, H-26), 0.78 (3H, d,  $J = 6.9$  Hz, H-27), 1.56, 1.56 (each 1 H, m, H-28), 0.9 (3H, t,  $J = 6.9$  Hz, H-29).  $^{13}C$ -NMR ( $CDCl_3$ ):  $\delta$  35.7 (C-1), 33.9 (C-2), 199.8 (C-3), 123.8 (C-4), 171.9 (C-5), 32 (C-6), 29.7 (C-7), 35.6 (C-8), 53.8 (C-9), 38.6 (C-10), 21 (C-11), 39.6 (C-12), 42.4 (C-13), 55.8 (C-14), 24.2 (C-15), 32.9 (C-16), 56 (C-17), 12 (C-18), 17.4 (C-19), 36.1 (C-20), 18.7 (C-21), 34 (C-22), 26 (C-23), 45.8 (C-24), 28.2 (C-25), 19.8 (C-26), 19 (C-27), 23 (C-28), 11.9 (C-29).

**3 $\alpha$ ,21 $\beta$ -Dimethoxy- $\Delta$ 14-serratene (C3)**. (EI-MS)  $m/z$  470 ( $M^+$ ;  $C_{32}H_{54}O_2$ , 54), 455 (35), 438 (18), 423 (18), 234, (52), 221, (81), 189 (100), 149 (20), 135 (72), and 147 (43). The  $^1H$ -NMR ( $CDCl_3$ ) results were as follows:  $\delta$  0.93 (3H, s, H-23-Me), 0.65 (3H, s, H-24-Me), 0.72 (3H, s, H-25-Me), 0.78 (3H, s, H-26-Me), 0.80 (3H, s, H-28-Me), 0.92 (3H, s, H-29-Me), 0.93 (3H, s, H-30-Me), 3.33 (3H, s, 21-OMe), 2.03 (3H, s, H-3-OMe), 2.62 (1H, dd,  $J = 11.9$  and  $4.1$  Hz, H-3 $\alpha$ ), 5.28 (1H, brs, H-15).

**$\beta$ -Sitosterol (C4)**. The EI-MS  $m/z$  414 ( $M^+$ ;  $C_{29}H_{50}O$ , 91), 396 (43), 81 (85), 55 (100). The  $^1H$ -NMR ( $CDCl_3$ ) results were as follows:  $\delta$  1.13, 1.13 (2H, m, H-1), 1.58, 1.23 (2H, m, H-2), 3.5 (1H, m, H-3), 2.23, 1.97 (2H, m, H-4), 5.33 (1H, m, H-6), 2.15, 1.97 (2H, m, H-7), 1.48 (1H, m, H-8), 1.44 (1H, m, H-9), 1.51, 1.22 (2H, m, H-11), 1.56, 1.47 (2H, m, H-12),

1.41 (1H, m, H-14), 1.63, 1.46 (2H, m, H-15, H-16), 1.47 (1H, m, H-17), 1.04 (3H, m, H-18), 1.05 (3H, m, H-19), 1.64 (1H, m, H-20), 0.98 (3H, d,  $J = 6.9$  Hz, H-21), 1.25 (2H, m, H-22, H-23), 1.46 (1H, m, H-24), 1.81 (1H, m, H-25), 0.81 (3H, d,  $J = 4.8$  Hz, H-26), 0.89 (3H, d,  $J = 4.8$  Hz, H-27), 1.49 (2H, m, H-28), 0.90 (3H, m, H-29).  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  37.2 (C-1), 31.6 (C-2), 71.8 (C-3), 42.2 (C-4), 140.7 (C-5), 121.7 (C-6), 31.9 (C-7), 31.8 (C-8), 50.1 (C-9), 36.5 (C-10), 22.6 (C-11), 39.7 (C-12), 42.3 (C-13), 56.7 (C-14), 24.3 (C-15), 26 (C-16), 56 (C-17), 11.8 (C-18), 19 (C-19), 36.1 (C-20), 18.7 (C-21), 33.9 (C-22), 26 (C-23), 45.8 (C-24), 29.1 (C-25), 19.8 (C-26), 21 (C-27), 23 (C-28), 12 (C-29).

**Serrate-14-en-3 $\beta$ ,21 $\beta$ -diol (C5).** EI-MS  $m/z$  442 ( $\text{M}^+$ ;  $\text{C}_{30}\text{H}_{50}\text{O}_2$ , 33), 427 (29), 409 (16), 391, 220 (26), and 207 (100). The  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ ) results were as follows:  $\delta$  0.94 (3H, s, H-23-Me), 0.74 (3H, s, H-24-Me), 0.77 (3H, s, H-25-Me), 0.81 (3H, s, H-26-Me), 0.91 (3H, s, H-28-Me), 1.02 (3H, s, H-29-Me), 1.06 (3H, s, H-30-Me), 3.17 (1H, dd,  $J = 11.7$  and 4.1 Hz, H-3- $\beta$ ), 3.43 (1H, brs, H-21- $\beta$ ), and 5.30 (1H, brs, H-15).  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  38.5 (C-1), 25.3 (C-2), 78.9 (C-3), 38.1 (C-4), 55.7 (C-5), 18.9 (C-6), 45.1 (C-7), 38.9 (C-8), 56.8 (C-9), 35.9 (C-10), 25.4 (C-11), 27.1 (C-12), 62.8 (C-13), 138.5 (C-14), 122 (C-15), 24 (C-16), 43.3 (C-17), 37.1 (C-18), 31.2 (C-19), 27.5 (C-20), 76.2 (C-21), 37.4 (C-22), 15.7 (C-23), 19.8 (C-24), 28.1 (C-25), 15.4 (C-26), 56.2 (C-27), 13.3 (C-28), 27.7 (C-29), 21.8 (C-30).

### GC-MS Analysis

Gas chromatography-mass spectrometry (GC-MS) data were collected with the instrument procured from Shimadzu, Kyoto, Japan, with the following conditions: column temperature from 50 °C (1 min) to 320 °C at 5 °C/min; injection temperature of 250 °C; detection temperature of 320 °C; acquisition mass range of 50 to 800 amu using helium as the carrier gas. The specification of column: DB-1 capillary column (30  $\times$  0.25 mm inner diameter and 0.25  $\mu\text{m}$ ; GL Sciences, Tokyo, Japan). The  $^{13}\text{C}$  and  $^1\text{H}$  nuclear magnetic resonance (NMR) were determined by a JEOL ECZ-400 spectrometer (JEOL, Tokyo, Japan). Then, the NMR spectra were recorded using standard JEOL pulse sequences at 400 MHz and 100 MHz for  $^1\text{H}$  and  $^{13}\text{C}$ , respectively.

### Antifungal Activity

The toxicity of the isolated compounds against the growth of *P. chrysosporium* were investigated through literature of Lukmandaru (2013). Exactly 1 mg/ml concentration of each sample was prepared and a solution of 300  $\mu\text{l}$  was placed on the surface of 20 ml PDA medium with 12.3 mg/  $\text{cm}^2$  concentration in a 9 cm diameter petri dish. The extract solution was allowed to stand for 1 h air dry before inoculation. The blank was performed to the sample solvent without extract and each sample was conducted in three replications. The lapachol analytical standard (Aldrich, Germany) was used in this assay as a positive control. Then, the growth rate of sample was calculated using equation 1:

$$\text{Growth rate (\%)} = A_1 / A_0 \times 100\% \quad (1)$$

Where  $A = \pi \times (d/2)^2$ ,  $d$  = diameter of sample growth,  $A_0$  is growth rate of blank and  $A_1$  is growth rate of sample.

### Statistical Analyses

One-way analysis of variance (ANOVA) were performed on the results of the growth rate using SPSS version 20 (IBM, New York, USA) with 95% confidence level. Significant results were further subjected to post-test Tukey HSD.

### Results and Discussion

#### Extractive Content and Constituents of *n*-hexane Extract

The extraction from the *P. merkusii* bark by *n*-hexane produced 1.59 g of extractive content on the basis of dry bark sample. In comparison, the extractive content of *P. merkusii* bark (0.16%) was lower than previous works i.e *P. echinata* bark of 2.6%, *P. taeda* of 1.7% (Fengel and Wegener 1989), and *P. pinea* of 2.1% (Nunes *et al.* 1999). According to Masendra *et al.* (2018a), the constituent of *n*-hexane extracts contained serratene triterpenes and sterols. The chromatogram of *n*-hexane extract of *P. merkusii* in Figure 2, showed that  $\beta$ -sitosterol (C4) was the major compound followed by 3 $\beta$ -methoxyserrate-14-en-21-one (C1). However, stigmast-4-en-3-one (C2), 3 $\alpha$ ,21 $\beta$ -dimethoxy- $\Delta$ 14-serratene (C3) and serrate-14-en-3 $\beta$ ,21 $\beta$ -diol (C5) were present in low concentration.

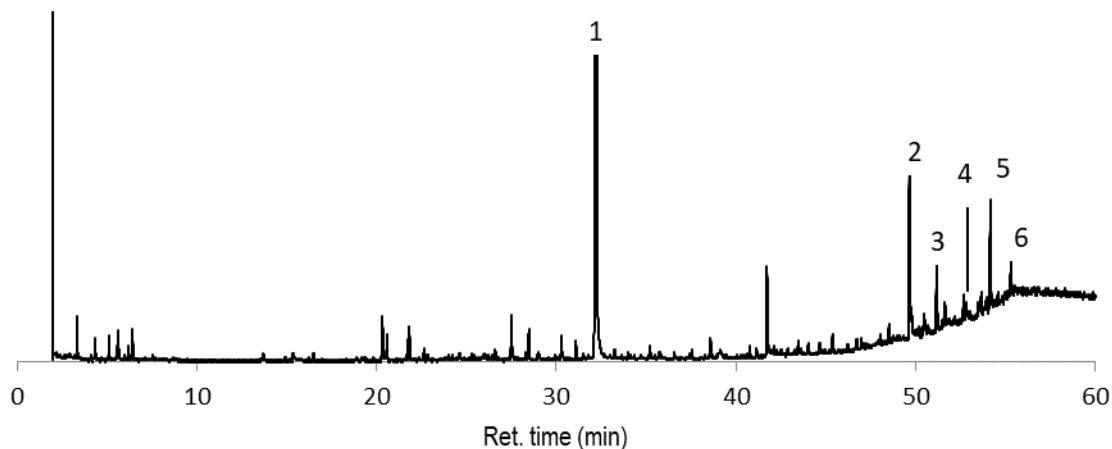


Figure 2. Chromatogram of *n*-hexane extract of *P. merkusii* bark; 1. Internal standard (heneicosane (ret. time: 32.26 min), 2.  $\beta$ -sitosterol (49.65 min), 3. Stigmast-4-en-3-one (51.17), 4.  $3\alpha,21\beta$ -dimethoxy- $\Delta^{14}$ -serratene (53.70), 5.  $3\beta$ -methoxyserrat-14-en-21-one (54.16), 6. Serrate-14-en- $3\beta,21\beta$ -diol (55.29).

#### Antifungal Activity of Isolated Compounds

The antifungal activity of isolated compounds was measured with reference to the growth rate of *P. chrysosporium*. The fungus growth on PDA medium as represented in Figure 3, showed a cycle shape and fungus spread from the center of petri dish to the surface of PDA. Also, the results of the one-way ANOVA showed significant difference ( $p < 0.01$ ) among the compound. The growth

rates of **C1-C3**, as shown in Figure 4, were 100% with no antifungal activity. However, there were significantly higher growth inhibition rates in  $\beta$ -sitosterol (C4) and serrate-14-en- $3\beta,21\beta$ -diol (C5) with inhibition of 45.5% and 60.2%, respectively, but still low compared with lapachol standard with 26.7%. Also, the positive control with lapachol was two times higher than  $\beta$ -sitosterol and serrate-14-en- $3\beta,21\beta$ -diol.

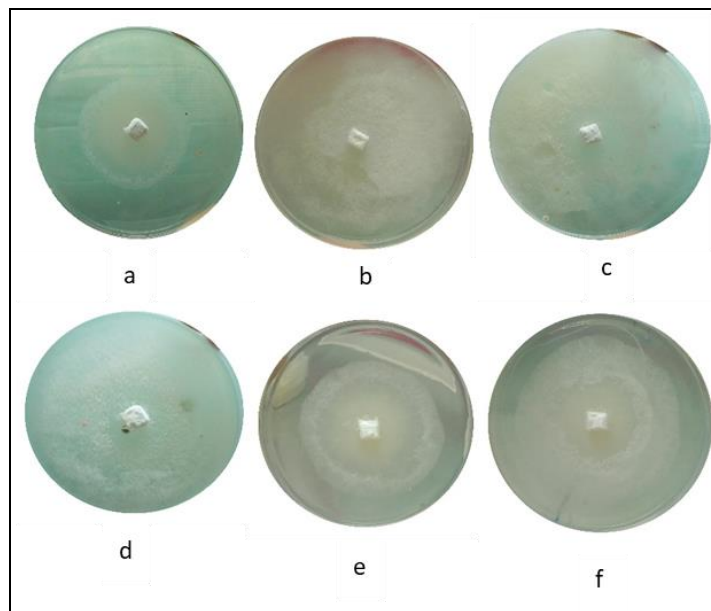


Figure 3. Growth rate of fungus performance of standard of lapachol (a), **C1** (b), **C2** (c), **C3** (d), **C4** (e), **C5** (f)

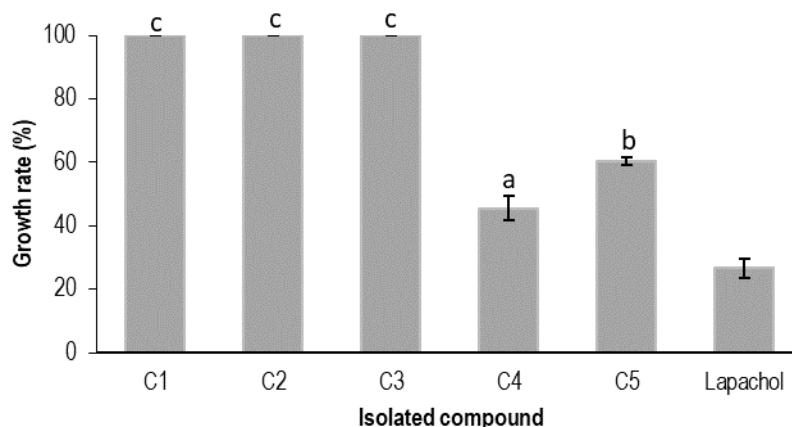


Figure 4. Antifungal activity of isolated compounds of *P. merkusii* bark. Different letter (a, b, etc.) indicates significant difference at 0.05 level by Tukey HSD test

In this research, no literature was found on the antifungal activity of triterpenoids and steroids from *P. merkusii* bark against *P. chrysosporium*. However, the antifungal activity of other compounds have been reported from other species such as 3b,6b,24-trihydroxyurs-12-en-27-oic acid from *Astilbe myriantha* showing strong antifungal activity against *Colletotrichum gloeosporioides* (Song *et al.* 2011), epilupeol acetate from *Ficus drupace* against *Aspegillus versicolor* (Yessoufoua *et al.* 2015), and  $\beta$ -sitosterol from *Senecio lyratus* against *Fusarium spp.* (Kiprono *et al.* 2000).

#### Correlation between Antifungal Activity and Chemical Structure of Isolated Compounds

It was observed that  $\beta$ -sitosterol and serrate-14-en-3 $\beta$ ,21 $\beta$ -diol affected the growth rate of *P. chrysosporium*. Also, among the isolated samples, only  $\beta$ -sitosterol and serrate-14-en-3 $\beta$ ,21 $\beta$ -diol have hydroxyl bond (-OH) in their structures as shown in Figure 1. This is an indication that the hydroxyl bond, which determine the lipophilicity and hydrophobicity of the compounds, affect the antifungal activity.

The hydroxyl bond was located in A and B rings in  $\beta$ -sitosterol, but in A and E rings in serrate-14-en-3 $\beta$ ,21 $\beta$ -diol. Hence, with  $\beta$ -sitosterol, the fungal growth rate was lower compared with serrate-14-en-3 $\beta$ ,21 $\beta$ -diol. This means that the hydroxyl position and hydrophobicity of A and B ring were stronger in inhibiting the growth of *P. chrysosporium* compared with the bond in A and E rings. In line with these findings, Sekine *et al.* (2009), and Zengin and Baysal (2014) also reported that the hydrophobicity and hydroxyl position of terpenes, latifolin, and the derivative products affected the antibacterial and antifungal activity by inhibiting the growth of brown- and white-rot. Additionally, a recent study by Konuk and Ergüden (2020) reported that the -OH position and hydrophobicity of phenolic terpenoids affected the

disruption of cell membrane integrity of *Saccharomyces cerevisiae*.

#### Ecological Role and the Potential Use of Triterpenoids and Steroids

The terpenoid compounds in *P. merkusii* bark was reported to play a vital role in preventing living tissues from chemical deterioration (Seki *et al.* 2012). In addition, the presence of triterpenoids and steroids in this bark, especially those with hydroxyl bond in their ring are more effective against *P. chrysosporium* (white-rot). Therefore, high concentration of triterpenoids and steroids in the bark of a tree protect its living tissues from damage due to fungal attacks.

Due to the impact of microorganisms such as bacterial and white-rot fungus attacks, there is need for further research on natural wood preservatives. The use of natural preservatives could reduce the negative effects of chemicals in the air. According to Tascioglu *et al.* (2013); Smith *et al.* (1989); and Lin *et al.* (2007), some plants extracts could be processed into alternative wood preservatives due to their actions against microorganisms' attacks. Hence, the accumulation of triterpenoids and steroids in the bark of *P. merkusii*, could be used as alternative wood preservatives.

#### Conclusion

Three triterpenoids (3 $\beta$ - methoxyserratt-14-en-21-one, serrate-14-en-3 $\beta$ ,21 $\beta$  -diol, and 3 $\alpha$ ,21 $\beta$ - dimethoxy- $\Delta$ 14-serratene) and two steroids ( $\beta$ -sitosterol and stigmast-4-en-3-one) were isolated and identified from the bark of *P. merkusii*. Among all these isolated samples,  $\beta$ -sitosterol and serrate-14-en-3 $\beta$ ,21 $\beta$  -diol showed antifungal activity with growth inhibition rates of 45.5% and 60.2%, respectively. However, the hydrophobicity and presence of hydroxyl bond in  $\beta$ -sitosterol and serrate-14-en-3 $\beta$ ,21 $\beta$  -diol affected the antifungal activity. Hence, there is need for further research

on the correlation between bioactivity and chemical structure of these two compounds with other fungi groups.

### Acknowledgments

This work was supported by BIDIK MISI scholarship. We thank to Mr. Sukmono Edwi Susanto (Perhutani enterprise) for providing research materials.

### References

- Alfredsen, G.; H. Solheim; R. Slimestad. 2008. Antifungal Effect of Bark Extracts from Some European Tree Species. *European Journal of Forest Research* 127: 387–393.
- Fengel, D.; G. Wegener. 1989. *Wood: Chemistry, Ultrastructure, Reactions*. Berlin: Walter de Gruyter.
- Kiprono, P.C.; F. Kaberia; J.M. Keriko; J.N. Karanja. 2014. The in vitro Anti-Fungal and Anti-Bacterial Activities of  $\beta$ -Sitosterol from *Senecio lyratus* (Asteraceae). *Zeitschrift für Naturforschung C* 55(5-6): 485–488.
- Konuk, H.B.; B. Ergüden. 2020. Phenolic –OH Group is Crucial for the Antifungal Activity of Terpenoids Via Disruption of Cell Membrane Integrity. *Folia Microbiologica* 65(4): 775-783. doi:10.1007/s12223-020-00787-4.
- Lin, C.Y.; C.L. Wu; S.T. Chang. 2007. Evaluating the Potency of Cinnamaldehyde as a Natural Wood Preservative. Stockholm: International Research Group Wood Preservation.
- Lomeli-Ramírez, M.G.; H. Dávila-Soto; J. Silva-Guzmán; H.G.O. Ruíz; S. García-Enriquez. 2016. Fungitoxic Potential of Extracts of Four *Pinus* spp. Bark to Inhibit Fungus *Trametes versicolor* (L. ex. Fr) Pilát. *BioResources*, 11(4): 10575-10584.
- Lukmandaru, G. 2013. Antifungal Activities of Certain Components of Teak Wood Extractives. *Jurnal Ilmu Teknologi Kayu Tropis* 11(1): 11-18.
- Masendra, T. Ashitani; K. Takahashi; G. Lukmandaru G. 2018a. Lipophilic Extractives of Inner and Outer Barks from Six Different *Pinus* Species Grown in Indonesia. *Journal of Forestry Research* 29(5): 1329-1336.
- Masendra, T. Ashitani; K. Takahashi; G. Lukmandaru G. 2018b. Triterpenoids and Steroids from the Bark of *Pinus merkusii* (Pinaceae). *BioResources* 13(3): 6160-6170.
- Masendra, T. Ashitani; K. Takahashi; M. Susanto; G. Lukmandaru. 2019. Hydrophilic Extracts of the Bark from Six *Pinus* Species. *Journal of The Korean Wood Science and Technology* 47(1): 80-89.
- Masendra, B.A.V. Purba; G. Lukmandaru. 2020. Methanol Soluble Extractives of *Pinus merkusii* bark and its Antioxidant Activity. IOP Conference Series: Materials Science and Engineering 935. Bogor, Indonesia.
- Nunes, E.; T. Quilho; H. Pereira. 1999. Anatomy and chemical composition of *P. pinea* L. bark. *Annals of Forest Science* 56: 479–484.
- Oliveira, L.S.; A.L.B.D. Santana; C.A. Maranhão; R.C.M. Miranda; V.L.A.G. Lima; S.I. Silva; M.S. Nascimento; L. Bieber. 2010. Natural Resistance of Five Woods to *Phanerochaete chrysosporium* Degradation. *International Biodeterioration & Biodegradation* 64: 711-715.
- Özgenç, Ö.; S. Durmaz; Ü.C. Yildiz; E. Erişir. 2017. A Comparison Between some Wood Bark Extracts, Antifungal Activity. *Kastamonu University Journal of Forestry Faculty* 17(3): 502-508.
- Pásztor, Z.; I.R. Mohácsiné; G. Gorbacheva; Z. Börcsök. 2016. The Utilization of Tree Bark. *BioResources* 11(3): 1-30.
- Seki, K.; K. Orihashi; M. Sato; M. Kishino; N. Saito. 2012. Accumulation of Constitutive Diterpenoids in the Rhytidome and Secondary Phloem of the Branch Bark of *Larix gmelinii* Var. Japonica. *Journal of Wood Science* 58, 437-445.
- Sekine, N.; T. Ashitani; T. Murayama; S. Shibutani; S. Hattori; K. Takahashi. 2009. Bioactivity of Latifolin and Its Derivatives Against Termites and Fungi. *Journal of Agriculture Food Chemistry* 57(13): 5707-5712.
- Shreaz, S.W.; A. Wani; J.M. Behbehani; V. Raja; M. Irshad; M. Karched; I. Ali; W.A. Siddiqi; L.T. Hun. 2016. Cinnamaldehyde and Its Derivatives, a Novel Class of Antifungal Agents. *Fitoterapia* 112: 116-131.
- Smith, A.L.; C.L. Campbell; D.B. Walker; J.W. Hanover. 1989. Extracts from Black Locust as Wood Preservatives, Extraction of Decay Resistance from Black Locust Heartwood. *Holzforshung*, 43(5): 293-296.
- Song, Q.Y.; W.Y. Qi; Z.M. Li; J. Zhao; J.J. Chen; K. Gao. 2011. Antifungal Activities of Triterpenoids from the Roots of *Astilbe myriantha* Diels. *Food Chemistry* 128: 495-499.
- Subhisha, S.; A. Subramoniam. 2005. Antifungal Activities of a Steroid from *Pallavicinia lyellii*, a Liverwort. *Indian Journal of Pharmacology* 37(5): 304-308.
- Tascioglu, C.; M. Yalcin; S. Sen; C. Akcay. 2013. Antifungal Properties of some Plant Extracts Used as Wood Preservatives. *International Biodeterioration & Biodegradation* 85: 23-28.
- Wijayanto, A.; S. Dumarçay; C. Gérardin-Charbonnier; R.K. Sari; W. Syafii; P. Gérardin. 2015. Phenolic and Lipophilic Extractives in *Pinus merkusii* Jungh. et de Vries Knots and Stemwood. *Industrial Crops and Products* 69: 466-471.
- Wittstock, U.; J. Gershenzon. 2002. Constitutive Plant Toxins and Their Role in Defense Against Herbivores and Pathogens. *Current Opinion in Plant Biology* 5(4): 300–307.
- Yessoufou, K.; H.O. Elansary; E.A. Mahmoud; K. Skalicka-Wozniak. 2015. Antifungal, Antibacterial and Anticancer Activities of *Ficus drupacea* L. Stem Bark Extract and Biologically Active Isolated Compounds. *Industrial Crops and Products* 74: 752-758.

Zengin, H.; H.B. Ayse. 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):17733-17798.

Masendra, Brandon Aristo Verick Purba, Ganis Lukmandaru  
Department of Forest Products Technology, Faculty of  
Forestry, Universitas Gadjah Mada,  
Jl. Agro No.1, Bulaksumur, Yogyakarta 55281, Indonesia  
Tel. : +6274 550541  
Fax. : +6274 550541  
Email : glukmandaru@ugm.ac.id

# WOOD RESEARCH JOURNAL

Volume 11, Number 1 & 2, 2020

## AUTHOR INDEX

### A

Aditya Aji Pratama 48  
Aisyah Kusumadewi 35  
Alberto Fernando Mussana 12  
Almaratush Schoolichah RIdio 27  
Andrade Femando Egas 12

### B

Barbara Ozarska 1  
Barbara Ozarska 41  
Bernadinus Herbudiman 53  
Brandon Aristo Verick Purba 20  
Brandon Aristo Verick Purba 65

### D

Delima 53  
Denny Irawati 27  
Dian Anggraini Indrawan 1

### F

Fatimah Zulaikha Wati 20  
Fatra Valahatul Ihda 20  
Fuad Sumantri 20

### G

Ganis Lukmandaru 20  
Ganis Lukmandaru 27  
Ganis Lukmandaru 58  
Ganis Lukmandaru 65  
Gustan Pari 1  
Gustan Pari 41

### J

### M

Mārcio Pereira Da Rocha 12  
Masendra 20  
Masendra 27  
Masendra 65

### N

Narciso Fernando Bila 12  
Nobuaki Hattori 48

### P

Peter Vinden 41

### R

Ratih Damayanti 1  
Ratih Damayanti 41  
Rini Pujiarti 35  
Rizki Arisanda 20  
Rosilani Trianoksi 12

### S

Setsuko Iwakiri 12

### T

# WOOD RESEARCH JOURNAL

Volume 11, Number 1 & 2, 2020

## KEYWORDS INDEX

### A

Anti – termite 58  
Antifeedant 58  
Antifungal 65  
Antioxidant 35

### B

Borax 48  
Boric acid 48  
Boring 12  
Brachystegia *spiciformis* 12  
Bubble test 41  
Bark 65

### C

Cardamom oils 35  
Chemical composition 35  
Clone 1  
Coloration 20

### D

Distillation time 35  
DPPH activity 27

### E

Extractives 1  
Extractives 27

### F

Fire resistance 48

### H

Heartwood 41  
Higher value – added products 12

### M

*Machrophyllia* 20  
Mahogany – wood 48  
Milling 12

### N

Neoflavonoid 27

### P

Permeability 41  
Physico – chemical properties 35  
Phytomedicine 27  
*Pinus merkusii* 65  
Planning 12  
Polyphenols 20  
Polysaccharides 20  
Pedestrian bridge 53

### R

Rhizome 35

### S

Sapwood 41  
Shaping 12  
Sonokeling 27  
Squalene 58  
Steroids 65  
Strength 53  
Super teak 1  
Surface quality 12

### T

Teak 58  
Tearing 12  
Tectoquinone 58  
Timber bridge 53  
Transition zones 41  
Triterpenoids 65

### W

Water soaking 41  
Wet and dry sites 1  
White – rot 65

**I**

Impregnation 48  
INDEX KATA KUNCI  
Indonesia 1

**J**

*Julbernardia globiflora* 12

**L**

Leaf 35  
LVL 53

**Y**

Young – teak 41  
Young teak 1

José Reinaldo Moreira da Silva 12

Jugo Lic 1

Jugo Liic 41

**K**

Keisuke Ando 48

Krisdianto 1

Krisdianto 41

Tomy Listyanto 48

**W**

Wahyu Dwianto 1

**Y**

Yosafat Aji Pranata 53

# WOOD RESEARCH Journal

## Journal of Indonesian Wood Research Society

### Annals of the Wood Research Journal

Wood Research Journal is the official journal of the Indonesian Wood Research Society. This journal is an international medium in exchanging, sharing and discussing the science and technology of wood.

### Aims and Scope

The journal publishes original manuscripts of basic and applied research of wood science and technology related to Anatomy, Properties, Quality Enhancement, Machining, Engineering and Constructions, Panel and Composites, Entomology and Preservation, Chemistry, Non Wood Forest Products, Pulp and Papers, Biomass Energy, and Biotechnology. Besides that, this journal also publishes review manuscripts which topics are decided by the Editors.

### Imprint

WRJ is published by Indonesian Wood Research Society

**ISSN print: 2087-3840**

Electronic edition is available at:

<http://ejournalmapeki.org/index.php/wrj>

### Publication Frequency

Journal is published in one volume of two issues per year (April and October).

### Peer Review Policy

WRJ reviewing policies are: Every submitted paper will be reviewed by at least two peer reviewers. Reviewing process will consider novelty, objectivity, method, scientific impact, conclusion and references.

### General Remarks

Manuscripts will be accepted for publications are those discussing and containing results of research on wood science and technology, and reviews on specific topics, which are decided by the Editors and have not been published elsewhere. Authors are requested to correct the manuscripts accepted for publications as suggested by the Reviewers. Editors could change positions of Figures and Tables.

### Manuscripts Preparations

1. Manuscripts must be in English, typewritten using Word, Arial Narrow, single space, 3 cm of left and right margin and 2.5 cm of top and bottom margin of a Letter paper size. Title is printed with a font size of 14 pt, Authors are of 12 pt, and Text is of 10 pt.
2. Manuscripts should be checked for spelling and grammar by a native speaker.
3. Manuscripts compositions:
  - 3.1. Title
  - 3.2. Complete name of Authors
  - 3.3. Abstract
  - 3.4. Key words
  - 3.5. Texts:
    - Introduction
    - Materials and Methods
    - Results and Discussion
    - Conclusions (and Suggestions)
    - References
    - Name and complete address of Authors
    - Appendix
4. Other rules:
  - 4.1. Names of wood are followed by Botanical Name.
  - 4.2. Values between are written using this symbol (~), e.g. 3.75 ~ 8.92%.
  - 4.3. Editors could modify Figures without changing their substantial meaning.
  - 4.4. References are arranged from A to Z.
  - 4.5. References in text are written as this example: (Palomar *et al.* 1990; Arancon 1997).
  - 4.6. Examples of writing of References: Altschul, S.F.; T.L. Madden; A.A. Schäffer; J. Zhang; Z. Zhang; W. Miller; D.J. Lipman. 1997. Gapped BLAST and PSI-BLAST: A New Generation of Protein Database Search Programs. *Nucleic Acids Res.* 25: 3389-3402.

### Editorial Address

Research Center for Biomaterials, Indonesian Institute of Sciences

Jl. Raya Bogor Km 46, Cibinong, Bogor 16911, Indonesia

Tel/Fax : +62-21-87914511/87914510

E-mail : [ejournal.wrj@gmail.com](mailto:ejournal.wrj@gmail.com)

Web-site : [www.ejournalmapeki.org](http://www.ejournalmapeki.org)

# WOOD RESEARCH Journal

Journal of Indonesian Wood Research Society

### Example of Table and Figure

Table 1. Effects of temperature on *in vitro* growth of seedlings.

Temp. (°C)	Shoot length (mm)	Number of leaf	Fresh weight (g)
25	59.2 ± 10.6 <sup>c</sup>	4.5 ± 0.8 <sup>a</sup>	0.29 ± 0.13 <sup>a</sup>
27	88.5 ± 9.3 <sup>a</sup>	4.8 ± 0.9 <sup>a</sup>	0.40 ± 0.12 <sup>a</sup>
29	75.0 ± 11.1 <sup>b</sup>	3.8 ± 0.6 <sup>a</sup>	0.30 ± 0.07 <sup>a</sup>

Note: Values (average ± standard deviation) with different letters are statistically significant according to Tukey's multiple comparison test. Data were recorded after 4 weeks of culture. MS medium was used as a basal medium without any PGRs. Number of sample = 10.

Source: Chujo *et al.* 2010.

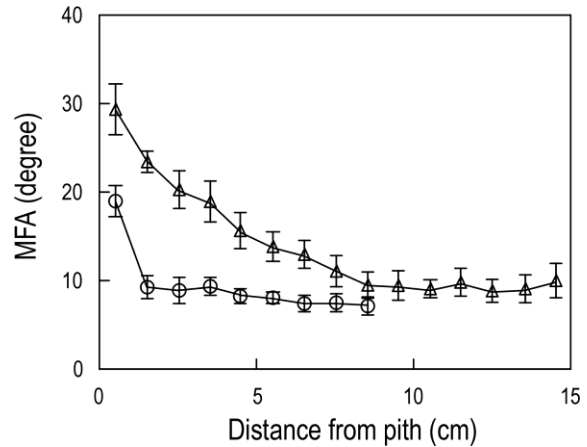


Figure 3. Radial variation of microfibril angle of the S2 layer in tracheid. Open circle, *Agathis sp.*; open triangle, *Pinus insularis*; Bars indicate the standard deviation. (Source: Ishiguri *et al.* 2010)