



**Faculty of Resource Science and Technology**

**Bioactivities and Chemical Composition of Naturally Durable Heartwood  
Extractives**

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Bioactivities and Chemical Composition of Naturally Durable Heartwood  
Extractives

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A thesis submitted

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## DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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## ABSTRACT

Wood durability varies within and between the species due to the existence of toxic extractives. Wood extractives are known to affect the wood resistance against biological degradation agents such as fungi, insects and marine borers. This study was carried out to determine (i) the natural durability of *Neobalanocarpus heimii* (King) P.S. Ashton, *Eusideroxylon zwageri* Teijsm & Binn, and *Potoxylon melagangai* (Symington) Kosterm, towards fungal decay and termite attack with and without extractives content, (ii) to identify and characterize the chemical compositions and constituents of the soluble fractions of hexane, dichloromethane and methanol from *N. heimii*, *E. zwageri*, and *P. melagangai* extractives, (iii) the determine quantities of extractable extractives compounds from different species using different solvents, and (iv) to assess wood durability from methanol extractives of *N. heimii*, *E. zwageri*, and *P. melagangai* to *D. polyphylla*. Heartwood samples were cut and ripped to obtain woodblocks of 1 × 1 × 1 cm cube. Some of the woodblocks underwent extraction and some were further ground to form a wood meal. The extraction of wood cubes was carried out using a Soxhlet extractor with a different solvent which was 95% Ethanol + Toluene, 95% Ethanol, 95% Methanol and finally hot distilled water in order of increasing polarity. Soil block and termite resistance tests were conducted on both extracted and unextracted *N. heimii*, *E. zwageri*, *P. melagangai* and *D. polyphylla* woodblocks. Woodblocks were exposed to *Schizophyllum commune* Fr. (white rot), *Trametes versicolor* (L.) Lloyd (white rot), and *Coniophora puteana* (Shum.: Fr.) P. Karst (brown rot), while *Coptotermes gestroi* Wasmann was used for the termite resistance test. Weight losses due to decay and termite attacks were determined after thirty-two weeks and three weeks of exposure, respectively. Wood meals were subjected to extraction using solvents of increasing polarity, firstly hexane, dichloromethane and methanol. The methanol-soluble

extract was used to treat *Dyera polyphylla* (Miq.) Steenis woodblocks. The removal of extractives content significantly reduced the durability of *N. heimii*, *E. zwageri*, and *P. melagangai*. Woodblocks were more susceptible to termite attack after the extraction of their extractive content. The results from the wood treatment test showed that methanol-soluble extractives successfully treat *D. polyphylla* woodblocks and at the concentration of 10% it provides excellent protection against wood decay fungi. Gas chromatography/mass spectroscopy (GC/MS) analyses showed that up to 100 compounds were detected in hexane-soluble and DCM-soluble extractives of *N. heimii*, *E. zwageri* and *P. melagangai*. There were more compounds from 103 to 144 were detected in methanol-soluble extractives of *N. heimii*, *E. zwageri* and *P. melagangai*. The major compounds detected in hexane-soluble and dichloromethane-soluble extractives of *N. heimi* are  $\alpha$ -Linolenic acid (5.10%) and 6-Ethyl-3-decanol (6.13%). Major compound detected in hexane-soluble and dichloromethane-soluble extractives of *E. zwageri* was Isoelemicin (46.81% and 46.83%). Compound 2-Ethoxycarbonyl-3-methyl-4-azafluorenone, 2-fluorenylimime (40.21%) was the main compound in hexane-soluble and dichloromethane-soluble extractives of *P. melagangai*. Phenol, and 4-methoxy-6-prop-2-enyl-1,3-benzodioxol-5-ol were the major compounds detected in methanol-soluble extractives of *N. heimii*, *E. zwageri* and *P. melagangai*, respectively. There are numerous compounds present in *N. heimii*, *E. zwageri* and *P. melagangai* extractives that are biologically active, protecting them from decay.

**Keywords:** Natural durability, wood extractives, soil block test, wood treatment, gas chromatography-mass spectrometry (GC-MS) analysis

**Komposisi Kimia Ekstraktif Teras Kayu Beberapa Spesis Kayu yang Mempunyai Sifat Ketahanan Semula jadi**

**ABSTRAK**

*Ketahanan semulajadi kayu berbeza mengikut spesies dan sesama spesies ia bergantung kepada kandungan ekstraktif toksik yang terdapat di dalamnya. Kandungan ekstraktif dipercayai memberi ketahanan semulajadi kayu kerana ia memberi rintangan terhadap agen-agen biodegradasi seperti kulat, serangga dan penggerek laut. Kajian ini dijalankan untuk menentukan (i) ketahanan semulajadi kayu Neobalanocarpus heimii (King) P.S. Ashton, Eusideroxylon zwageri, Teijsm & Binn, dan Potoxylon melagangai (Symington) Kosterm, pada blok kayu yang telah diekstrak dan tanpa diekstrak terhadap serangan kulat reput, (ii) mengenalpasti komposisi dan sebatian kimia daripada larutan heksana, diklorometana dan metanol daripada ekstraktif N. heimii, E. zwageri, dan P. melagangai (iii) menentukan kuantiti ekstraktif yang boleh diekstrak melalui beberapa pelarut, daripada spesis yang berbeza, dan (iv) menilai kemungkinan meningkatkan ketahanan kayu yang tidak tahan setelah memindahkan ekstraktif methanol dari N. heimii, E. zwageri, dan P. melagangai kepada D. polyphylla. Sampel-sampel dari bahagian teras kayu dipotong dan diracik untuk mendapatkan blok kayu bersaiz 1 × 1 × 1 sm kiub. Beberapa blok kayu ini menjalani pengekstrakan dan ada yang dikisar menjadi serbuk kayu. Pengekstrakan blok kayu dijalankan dengan menggunakan ekstraktor Soxhlet bersama pelarut yang berbeza kepolarannya, iaitu 95% Etanol + Toluena, 95% Etanol, 95% Metanol serta air suling. Ujian blok tanah dan ujian kerentangan anai-anai dijalankan bagi kedua-dua blok kayu yang diekstrak dan tanpa diekstrak dari N. heimii, E. zwageri, P. melagangai dan D. polyphylla. Blok kayu didedahkan kepada Schizophyllum commune Fr. (white rot), (kulat reput putih), Trametes versicolor (L.) Lloyd (kulat reput putih) dan*

Coniophora puteana (Shum.: Fr.) P.Karst (kulat reput koko), manakala Coptotermes gestroi Wasmann, digunakan untuk ujian kerentangan anai-anai. Kehilangan berat akibat reput dan seranga ana-anaai masing-masingnya ditentukan setelah menjalani 32 minggu dan tiga minggu pendedahan. Serbuk kayu menjalani pengekstrakan menggunakan pelarut yang meningkat kepolarannya dimulai dengan heksana, diklorometana dan metanol. Ekstrak larut dalam metanol digunakan untuk merawat blok kayu D. polyphylla (Miq.) Steenis. Keputusan menunjukkan pengekstrakan ekstrakatif menurunkan ketahanan semulajadi kayu N. heimii, E. zwageri, dan P. melagangai dengan signifikan. Blok kayu juga lebih rentan kepada serangan anai-anai setelah pengekstrakan. Keputusan juga menunjukkan rawatan blok kayu D. polyphylla menggunakan ekstrak metanol bejaya mengelakkan blok kayu tersebut dari reput pada kepekatan 10%. Analisis kromatografi gas menunjukkan terdapat sehingga 100 sebatian dikenalpasti dalam ekstrak heksana and ekstrak diklorometana (DCM). Terdapat dari 103 ke 144 sebatian dikesan dalam ekstrak metanol dalam N. heimii, E. zwageri, dan P. melagangai. Sebatian utama dikesan dalam heksana dan DCM ekstrak masing-masingnya ialah  $\alpha$ -Linolenic acid (5.10%) dan 6-Ethyl-3-decanol (6.13%). Sebatian utama dikesan dalam heksana dan DCM ekstrak dari E. zwageri ialah Isoelemicin (46.81%). Sebatian 2-Ethoxycarbonyl-3-methyl-4-azafluorenone, 2-fluorenylimime (40.21%) merupakan sebatian utama dalam heksana dan DCM ekstrak dari ekstrakatif P. melagangai. Fenol, dan 4-methoxy-6-prop-2-enyl-1,3-benzodioxol-5-ol merupakan sebatian utama masing-masingnya dikesan dalam ekstrak metanol N. heimii, E. zwageri, dan P. melagangai. Terdapat pelbagai sebatian yang ada dalam ekstrakatif N. heimii, E. zwageri, dan P. melagangai yang mempunyai bioaktiviti yang boleh melindungi kayu tersebut dari reput.

**Kata kunci:** Ketahanan semulajadi, ekstrakatif kayu, ujian blok tanah, rawatan kayu, analisis kromatografi gas-spektrometri jisim (GC-MS)



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## LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
ASTM	American Society for Testing and Materials
CGS	Centre for Graduate Studies
FRIM	Forest Research Institute of Malaysia
L	Litre
MEA	Malt Extract Agar
Mm	Millimetre
UNIMAS	Universiti Malaysia Sarawak
°C	Degree celsius

# CHAPTER 1

## INTRODUCTION

### 1.1 Study Background

Forest provided humans with vital elements such as timbers for multiple uses, non-timber forest products such as bamboo, ‘damar’ and rattan, the wild fruits such as ‘petai’ (*Parkia spp.*), and also medicinal plants including remedies against disease. The tropical rainforest is a source of chemicals in the pharmaceutical industries, potentially developing new drugs (VanSeters, 2003). A complex ecosystem exists primarily involving forest trees, which in turn act as supporters and defenders of life systems on the earth. A critical environmental service is provided by the forest, such as regulating water flow into our rivers or streams, and regulating climate changes, helps clean and cooler the air during hot days, acts as a superb sound absorber, and helps in declining soil erosion (Hamid & Abd Rahman, 2016). Timber is undeniably the dominant tangible product of the forest. According to Mark et al. (2014), timber is extensively used for making furniture, mainly used for building structures and various other purposes depending on its properties. One of the major properties that determine timber utilization is its durability (Mark et al., 2014). Dimensional stability determines the use and variability of wood and is one of wood's important natural durability features (Devi, 2004; Pandey, 2009).

Naturally durable woods have multiple uses as commercial timber. The natural durability of natural decay resistance is an essential property of timber. Timber can resist decay by wood-destroying fungi. The durable woods have the natural resistance to decay due to fungi attacks but also to insects, marine borers and weathering. Many kinds of wood have been evaluated in field and laboratory tests about their natural durability, especially

commercial woods (Tascioglu et al., 2012). The performance and adaptability of the wood depends on its structure and properties, which are the workability, strength, the combined strength and light weight, hardness, its resistance to decay, its permeability, the heat and sound insulation, its response to sound vibration, the odour, the colour and grain, its strength under the weight shock, its plasticity and resiliency and the chemical characteristics of the woods (Sharpe et al., 1992).

Natural durability or decay resistance of wood is commonly attributed to the fungi toxic extraneous materials or extractives deposited during heartwood formation (Fengel & Wegener, 1989; Eaton & Hale, 1993). Some factors have been reported to be responsible for the natural durability of wood other than the presence of toxic extractives in the heartwood, and these include lignin type and quantity (Yamamoto & Hong, 1988; Yamamoto & Hong, 1989), heartwood permeability, nitrogen content, presence or absence of mineral deposits as well as the rate of growth (Singh et al., 1992). The durability of wood also depends on the surrounding environment, such as the presence of sufficient moisture, oxygen, and a suitable temperature is necessary for the survival as well as the continuous growth of wood-destroying organisms (Eaton & Hale, 1993).

The highly durable tropical hardwood species usually contain high levels of extractives, show good dimensional durability and stability and have been used extensively as construction materials. The durability of tropical hardwood is attributed to the toxicity and water-repellence of extractives composition (Xie et al., 2012). There are two important features of wood components, which are heartwood and sapwood. Heartwood differs from sapwood in terms of its darker colour, lower permeability towards substances and higher resistance towards decaying organisms. Heartwood located in the inner section of the wood,

therefore contains higher extractives content that response to a build-up of toxic by-products of chemical metabolism which result in the death of parenchyma cells, compared to sapwood which is located in the outer section of the wood (Taylor et al., 2002). Generally, the natural durability of sapwood is low, but for heartwood of some species, the resistance shown towards bio-deteriorating agents are remarkable (Taylor et al., 2006).

Extractives are a mixture of low-molecular-weight compounds either a lipophilic or hydrophilic compounds (McGinnis & Shafizaden, 1980; Sjöström, 1993) such as tannins, other polyphenolics, colouring matters, essential oils, fats, resins, waxes, gums and starch (Fernández et al., 2001). It can be extracted either using non-polar or polar solvents such as water, alcohol, acetone, benzene and ether. The type of extractives and its distribution vary with species, the location of the trees in stem or age (Goldstein, 1991; Zabel & Morrell, 1992; Suttie & Orsler, 1996). The concentration of extractives leads to a certain important role that gives wood its unique properties like colour (Gierlinger et al., 2002) and odour to wood. Odour is a result of aromatic compounds present in extractives (Shmulsky & Jones, 2011). In most wood, the heartwood is darker than sapwood due to random oxidation reactions of secondary metabolites. Meanwhile, there are no colour differences between heartwood and sapwood in some wood due to the absence of dark-coloured extractives. The colour of extractives varies from yellow, red and brown or sometimes colourless, and the concentration varies within trees. The Colour of wood changes when exposed to light or heat due to extractive oxidation.

This study determined the role of *Neobalanocarpus heimii* (King) P.S. Ashton (cengal), *Eusideroxylon zwageri* Teijsm & Binn (belian), and *Potoxylon melagangai* (Symington) Kosterm (melagangai), extractives and assessed in terms of their decay

resistance toward fungi and termite attack. The ability of these selected woods to resist decay without extractive was also observed in this study. The amount and extractives compound of the selected hardwood will be analysed using gas chromatography-mass spectrometry (GC-MS) and tested as natural-based preservatives toward non-durable wood, so that they are able to defend themselves against the microorganisms, thus making them suitable to be used for industrial purposes, such as panelling and decorative furniture. The extractives isolated from natural resistant heartwood and some plant species may provide pest control alternatives because they possess bioactive chemicals (Syafii & Yoshimoto, 1993).

## 1.2 Problem Statement

Little information is available regarding the role of extractives in *N. heimii*, *E. zwageri*, and *P. melagangai* natural durability. Besides, the chemical compounds responsible for their durability has not been extensively studied. Only these three species, among other thousand species exist in Tropical Forest was chosen because these species are among the strongest timber species in Malaysia, while these timber species are widely used as construction material in Malaysia (Menon et al., 2004). Furthermore, these three species are listed as endangered species (*N. heimii*) and vulnerable species (*E. zwageri*, and *P. melagangai*) in the IUCN Red list site and the studies on why these species are naturally durable are limited. Although wood extractives have been extracted from *N. heimii*, *E. zwageri*, and *P. melagangai*, minimal data are available regarding its chemical composition and constituent. The bioactivities of compounds obtained from wood extractives have hardly been investigated as well. What is/are the chemical responsible for the decay resistance of *N. heimii*, *E. zwageri*, and *P. melagangai*? Are the amount and chemical compounds the same or different? Do types of extractives differ in their bioactivities?

According to Subekti et al. (2018), the result shows *Eusideroxylon zwageri* high resistance to termite attack after radiation exposure. The study by Jusoh and Assim (2013), shows that both heartwood of *Eusideroxylon zwageri* and *Potoxylon melagangai*, high resistance to fungal decay after being extracted using acetone. Kim et al. (2006), stated that the heartwood of *Neobalanocarpus heimii* has a high degree of decay resistance due to its high extractive content. Among common compounds found from fractionated extractives were 5- octadecene, palmitic acid and 4-tetradecanol, while compounds highly degraded were 9-octadecene, 4-tetradecanol and 2- propenoic acid. Vanillin, 1-nitro-3,5-dimethoxyphenyl-ethylene, 2,4-dimethoxy-5,6-dimethylbenzaldehyde, benzenamine and 5-allyl-1,2,3-trimethoxybenzene compounds derived after wood exposure to *Pycnopus coccineus* and *Schizophyllum commune* (Jusoh & Assim, 2013). Syafii et al. (1987) reported that the high resistance of *E. zwageri* is obscured to the presence of eusiderin.

### **1.3 Research Objectives**

The main aim of this study was to determine the role of wood extractives in the natural durability of *N. heimii*, *E. zwageri*, and *P. melagangai*. The specific objectives of this study were:

- i. To determine the natural durability of *N. heimii*, *E. zwageri*, and *P. melagangai* towards fungal decay and termite attack with and without extractives content;
- ii. To identify and characterize the chemical compositions and constituents of the soluble fractions of hexane, dichloromethane and methanol from *N. heimii*, *E. zwageri*, and *P. melagangai* extractives;