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Research Article

# Antifungal Activities of *Neobalanocarpus heimii* (Cengal) Heartwood Extracts on *Trametes versicolor* and *Coniophora puteana*

**Nur Afiqah Manan[1], Ismail Jusoh[2], Furzani Pa'ee[1]***
1) Department of Technology & Natural Resources, Faculty of Applied Sciences & Technology, Universiti Tun Hussein Onn Malaysia (UTHM)Pagoh Campus , Pagoh Educational Hub, KM 1, Jalan Panchor, 84600 Panchor, Johor.
2) Universiti Malaysia Sarawak, Jalan Datuk Mohammad Musa, 94300 Kota Samarahan, Sarawak
* Corresponding author, email: furzani@uthm.edu.my

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## ABSTRACT

*Neobalanocarpus heimii* (Cengal) is from the family Dipterocarpaceae. It is a long-lasting wood that is also one of the most robust timbers in the world. This species is native to Peninsular Malaysia and southern Thailand**.** In this study, the Cengal heartwood was studied concerning the amount of water extractive content with antifungal properties from the *Neobalanocarpus heimii*. The dilution method was used to test the antifungal properties. Wood meals samples were subjected to the sequential extractive beginning with hexane followed by dichloromethane, methanol and water. The extracts were collected and underwent evaporation by using rotary evaporator to obtain pure crude extract. The antifungal activities were determined using agar dilution method. Two selected fungi *Trametes versicolor* (*T. versicolor*) and *Coniophora puteana* (*C. puteana*) were used. The antifungal index (%) which compares the diameter of the growth zone for the experimental plate and control plate was calculated. The total percentage of yield from *Neobalanocarpus heimii* was 0.28%. The highest antifungal index obtained for *Trametes versicolor* (*T. versicolor)* was 81.22%, while *C. puteana* was 43.24%. The crude extracts from *Neobalanocarpus heimii* were effective in inhibiting the growth of *Trametes versicolor* and *Coniophora puteana.*



## INTRODUCTION

The traditional toxic wood preservatives that create environmental hazards can be replaced by secondary metabolites of timber with antifungal properties and can be used as natural biodegradable fungicides. Antifungal refers to a chemical compound produced biosynthetically or synthetically that could destroy or usefully suppress the metabolism of various harmful microscopic organisms (Kawamura et al. 2010).

An abundance of naturally durable wood species demonstrates improved efficiency and durability in outdoor exposure without preservatives. When assessing and forecasting the efficiency of a naturally durable wood species, wood extractives are widely considered to be a contributing factor (Kirker et al. 2013). Wood extractives are the non-structural components of wood. It is often produced by the standing tree as defensive compounds to

environmental stresses and is typically concentrated in the heartwood (Taylor et al. 2002). In terms of wood extractives, extractive content varies greatly not only from tree to tree but also within an individual tree, and by employing various extraction methods, extractives from wood species with high natural resistance can be used to improve the durability of non-durable wood against insects, fungi, mould, and termites (Kadir & Hale 2019).

According to (Bernhoft 2010), plants generate bioactive compounds as secondary metabolites, a form of compound other than primary metabolites that are thought to aid plants in increasing their overall ability to thrive and resolve local challenges by enabling them to interact with their environment. The occurrence of wood-decay fungi species may affect factors such as the degree of decay, the amount of bark left on the log and the previous fungal population (Renvall 1995; Niemelä et al. 1995; Kruys et al. 1999). The community of decomposers can be affected in many ways by the internal wood characteristics partly related to the tree growth rates. A higher concentration of total nitrogen and amino acids was found in the fast-growing wood from *Pinus sylvestris* than slow-growing wood shown by (Sundberg et al. 1993). N and P concentrations are essential determinants of fungal growth rates (Edman et al. 2006), and this is also probably true for amino acids.

*Neobalanocarpus heimii* (*N. heimii* = Cengal) is well known throughout the Peninsular. According to (Symington et al. 2004), the most botanical difference of this species is in flower, having oblong anthers and short appendages quite unlike the oval anthers and filiform appendages of balanocarpus bed., 7 which is now considered a synonym within the type of hopea sections. *Neobalanocarpus heimii* comes from the family of dipterocarpaceae, one of Malaysia's heavy hardwoods' products. Furthermore, many dipterocarp plants have been found to contain a variety of terpenoids and sesquiterpenes, which is thought to act as a defence compound against fungi and insects (Kadir & Hale 2019). Yamamoto (1988) stated that Cengal has a durable wood with air -dry density ranging from over 915 to 980 kg/m$^3$ even under adverse conditions. According to Symington et al. (2004), Cengal is among the most robust timbers globally that is 50% stronger than teak and resistant to termite and fungi. In addition, high extractive contents in the heartwood of *N. heimii* give a high degree of decay resistance (Kim et al. 2006). It also offers high economic value due to its strength and durability. Thus, it produces a strong and naturally durable wood used for heavy construction, boats, buildings, bridges, to which strength is essential (Tnah et al. 2012). Furthermore, it can improve the cooling effect for Malaysia's green building which is designed to save energy consumption, to minimize the impact on climate change, and to reduce the rate at which we consume natural resources. Cengal has good thermal conductivity and resistivity and can circulate heat more effectively than Meranti (Mohamed et al. 2015).

## MATERIALS AND METHODS

### Sample collection

A sample of the heartwood of *Neobalanocarpus heimii* was obtained from Bentong, Pahang. 8.0 kg of fresh sample was collected and air-dried for two months from September to November 2017. The heartwood sample of Cengal was milled to a very fine homogenous composition and grounded into a fine powdery mixture (Hosseinihashemi et al. 2013).

### Solvent extraction

Extraction of wood was carried out using solvent extraction method according to the procedure described by (Chang et al. 1999) with slight modification. The wood meal of *Neobalanocarpus heimii* was placed in the 3000 mL separator funnel and immersed in hexane solvent (non-polar) for two weeks to remove the hexane-soluble compound from the samples. The wood meals in the separator funnel were evenly stirred with a glass rod twice a day every day for two consecutive weeks. The crude extract was drained and the solvent was subsequently replaced with dichloromethane (semi-polar), methanol and water (polar). The solvent for the extraction was replaced by a polar solvent from a non-polar one to get the pure crude of water extract from *Neobalanocarpus heimii* wood meals. The extract was evaporated into dryness using a vacuum rotary evaporator at 100˚C. Pure crude extracts were dried in an oven at 60˚C and weighed.

The collected crude extracts were tested to determine their antifungal properties. The percentage yield of the crude extracts was obtained from the weight of the sample before and after the extraction procedure. The percentage was calculated as follows (Chang et al. 1999):

$$Y\,(\%) = \frac{S_2}{S_1} \times 100\% \qquad (1)$$

Where:

Y = Yield percentage of pure crude

$S_1$ = Weight of wood sample before extraction

$S_2$ = Weight of crude extract

### Dilution method

The method that was used for the antifungal properties is the dilution method. In this method, 10.0 g of crude extract was diluted with distilled water to prepare the stock solution. This dilution was done in order to obtain final concentration of 50 mg/ml, 25mg/ml and 10 mg/ml. For each concentration, seven replications were prepared. Diluted water crude mixed with agar was used as treatment while agar without crude extract acted as a control. The diameter of growth of the mycelium on agar was observed and recorded after 6 days.

## Determination of Antifungal

### Preparation of fungi pure culture

The method used for fungi culture was described by (Sibero et al. 2016) with slight modification. 7.0 g of Malt Extract Agar (MEA) powder was weighed and dissolved in 200 mL of distilled water. MEA solution was stirred with a stirrer on the hotplate machine before autoclaving at 121˚C for two hours. MEA solution was poured onto the sterile disposable petri dishes and left to cool and solidify. Two selected fungi (*Trametes versicolor* and *Coniophora puteana*) were taken from stock culture and then transferred to Malt Extract Agar (MEA) and incubated at room temperature for 7 days. It was done aseptically in the lamina flow hood to prevent contamination. Fungi growth was checked frequently to make sure there was no contamination. The growing mycelia with a 0.5 cm diameter were then harvested and tested with different concentrations of crude heartwood extract from *N. heimii*.

### Antifungal analysis

The antifungal activity assay against of *T. versicolor* and *C. puteana* growth was carried out according to Özgenç et al. (2017). Each petri dish was filled with 15 mL of Malt Extract Agar (MEA) medium which contained the extracted sample at concentrations of 50, 25, and 10 mg/ml. The petri dish's centre was filled with a 1 cm disc of a tested fungi culture that had been growing for seven days. The colony's diameter was measured in centimetres after incubation. The following formula was used to calculate the antifungal index which was expressed as a percentage of inhibition (Chang et al. 1999),

$$\text{Antifungal index} = \left(1 - \frac{Da}{Db}\right) \times 100\% \quad (2)$$

Where Da is the mean diameter of growth zone in the experimental plate (cm) and Db is the mean diameter of growth zone in control plate (cm).

## RESULTS AND DISCUSSION

Crude extract of *Neobalanocarpus heimii* was obtained using successive extraction of different solvent polarity and the total percentage of water crude extract obtained was 0.28% with 19 g mass crude (Table 1).

The amount of extractive content found by percentage yield of water crude extractive obtained from *N. heimii* heartwood meal was 0.28%. The percentage obtained was too low. Low percentage yield might be caused by the temperature of the solvent extraction itself. According to Ahmad (2013), on previous study, removing extractives from sound wood in cold water was a prolonged process with an initial rate of 0.012% hour$^{-1}$ compared to hot water with an equivalent rate of 0.197% hour$^{-1}$. The duration of extraction does not play an essential role as a more extended extraction period resulted in a higher percentage of crude extract due to the longer time between the solvent and the solute. However, the solvent and sample would be stopped with extractive content after some duration, so excessive extraction was unnecessary. This is based on Fick's law of diffusion (Baldosano 2015).

**Table 1**. Percentage yield of crude extracts from N. heimii heartwood after extracted with hexane, dichloromethane, methanol, and distilled water.

| Wood meal sample (g) | Crude extract (%) |
|---|---|
| *Neobalanocarpus heimii* | 0.28 |

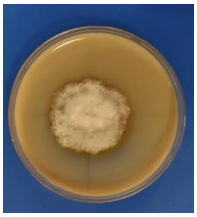
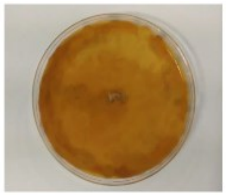
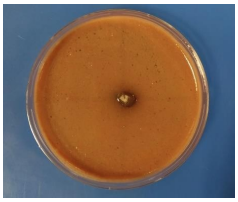
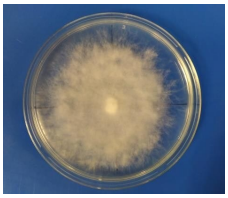
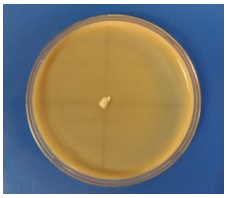
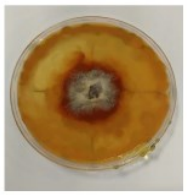
Among the three concentrations of crude extract used as seen in Table 2, the maximum antifungal index was 100% at the highest concentration 50 mg/ml for both fungi, *T. versicolor* and *C. puteana*. At a 25 mg/ml concentration, the antifungal index was 100% and 43.24% for *T. versicolor* and *C. puteana,* respectively. The record also shows that the concentration of crude extract at 25 mg/ml inhibits the growth of T. versicolor but is susceptible to *C. puteana*. When *T. versicolor* was tested using 10 mg/ml concentration, the antifungal index showed 81.22%. Low concentration allows the growth of fungi and the diameter of fungi growth would be increased due to the high susceptible enzyme of *T. versicolor* with the crude extract. This indicates that the antifungal activities depend on the concentration of extracts, the higher concentrations completely inhibited mycelial growth (Bopenga et al. 2020). In fact, decay activity of the timbers that caused by the fungi was reduced by increasing the concentrations of wood extracts due to compound in the extracts that slowed fungal attack and decreased weight loss in a susceptible wood species (Kadir & Hale 2019).

**Table 2.** Antifungal index of T. versicolor and C. puteana in different concentration of water crude extract from *N. heimii.*

| Concentration (mg/ml) | Antifungal index (%) | |
|---|---|---|
| **Control** | ***Trametes versicolor*** | ***Coniophora puteana*** |
| | 0 | 0 |
| **10** | 81.22 | 0 |
| **25** | 100 | 43.24 |
| **50** | 100 | 100 |

The effectiveness of extractives against wood-rotting fungus *T. versicolor* and *C. puteana* has been evaluated in bioassay for antifungal activity. The fungal growth on wood extract on agar medium was tabulated in Table 3. The result showed that a high concentration of heartwood extracts inhibits fungus growth for both types, *C. puteana* and *T. versicolor* compared to the control sample. The results of antifungal assays of *T. versicolor* and *C. puteana* were tested with different concentrations of water crude extracts from *N. heimii*. Saidan et al. (2020) reported that Cengal wood showed antifungal activities against *C. albicans* and *A. brasiliensis*. Furthermore, some researchers also found the extract of Cengal heartwood have effective termiticidal and fungicidal properties against subterranean termites (*C. curvignathus* and *C. gestroi*) and fungi (*T. versicolor*, *C. puteana*, and *L. sajor-caju*) (Kadir & Hale 2019). The fungi *T. versicolor* was able to grow at a concentration of 10 mg/ml (81.22%) because the concentration of the extract was not effective in inhibiting the growth of fungi.

**Table 3.** Antifungal activity of *T. versicolor* and *C. puteana* in three different concentrations of sample.

| Types of fungi | Concentration of sample | | | |
|---|---|---|---|---|
| | Control | 10 mg/ml | 25 mg/ml | 50 mg/ml |
| *Trametes versicolor* |  |  |  |  |
| *Coniophora puteana* |  |  |  |  |

According to Teeri (1997), White-rot fungi secrete one or more of three extracellular enzymes important for lignin degradation, such as lignin peroxidase, manganese-dependent peroxidase, and laccase. As a result, they can completely mineralize all cell wall polymers in hardwood decay. In addition, plant diversity affects extractive material variation. Alkaloids, terpenoids, condensed tannins, and a variety of other chemicals can be found in extractives, and they are responsible for natural durability (Taylor et al. 2002).

There is no growth of *C. puteana* recorded in the concentration of 10 mg/ml. This might be due to some error when handling the laboratory session. At a concentration of 25 mg/ml and 50 mg/ml, *T. versicolor* did not show a positive growth rate as the increase in concentration lead to the effective growth inhibition of fungi. The antifungal index of *C. puteana* showed 43.24% at a concentration of 25 mg/ml. The fungi could still grow because the enzyme secreted by this species of brown-rot fungi is still active to degrade the extractive contained in the MEA solution. Antifungal properties start to inhibit the growth of *C. puteana* at a 50 mg/ml concentration whereby the extractives had produced a toxic effect on the continued growth of fungi. Scheffer et al. (1966) stated that some researchers attempted to correlate the natural resistance of wood to fungal decay with several factors such as toxic wood extractives, structural features of wood, lignin and cellulose content of cell walls, depletion of reserve food materials, moisture content and nitrogen content of the wood. In spite of this they are vital in determining wood properties such as durability. The ability of fungi to degrade wood is determined by its chemical properties and structural features (Kadir & Hale 2019).

## CONCLUSION

This study was demonstrated to determine the amount of water extracts from *Neobalanocarpus heimii* and to show how crude extracts affected the growth decay of two fungi, *Trametes versicolor* and *Coniophora puteana*. Crude

extracts obtained from *Neobalanocarpus heimii* were 0.28%. Antifungal properties of *N. heimii* were shown through the inhibition of growth of both fungi by crude extracts according to its level of concentration.

The antifungal activities determined by the antifungal index revealed that at a concentration of 25 mg/ml, *Trametes versicolor* was inhibited. Crude extracts have the higher inhibitory ability on *Trametes versicolor* which starts to inhibit growth at a concentration of 25 mg/ml while *Coniophora puteana* starts to inhibit growth at a concentration of 50 mg/ml. This study has shown that water crude extracts from *N. heimii* contain a compound that inhibit the growth of *Trametes versicolor* (white rot) and *Coniophora puteana* (brown rot).

## AUTHORS CONTRIBUTION

N.A.M. carried out the research, wrote, and revised the article. I.J. conceptualised the central research idea and provided the theoretical framework. F.P. supervised the writing progress.

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## CONFLICT OF INTEREST

The authors agree that this research was conducted in the absence of any self-benefits, commercial or financial conflicts and declare the absence of conflicting interests with the funders.

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