Identifikasi Senyawa Ekstraktif Kayu Cendana Papua dan Potensinya Sebagai Pengusir Nyamuk

(Identification of Extractives Compounds from Cendana Papua Wood and Its Potential as Mosquito Repellent)

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Abstract

This research aimed to identify chemical compounds of Cendana Papua (*Flindersia sp*.) wood and to elucidate its potential utilization as a mosquito repellent. The experimental methods were qualitative phytochemical analysis, GCMS Pyrolisis analysis, and mosquito mortality test, which was conducted to investigate the anti-mosquito property of this wood species based on SNI 06-3566-1994. The results indicated that phytochemical analysis of Cendana Papua wood showed medical phytochemical compounds such as alkaloids, saponin, flavonoid, terpenoid, glycoside, and phenolic. GCMS pyrolisis analysis indicated that of 43 compounds found within this wood, butylated hydroxyanisole (5.37%), carbamic acid (5.30%), 2,4 imidazolidine Dione (2.13%), and hydrazine (1.23%) are active compounds which possibly responsible for mosquito repellent. The mosquito mortality test showed that the composition of 50:50 for sawdust and coconut shells had the highest mortality rate (55.6%). The mortality rate indicated that this wood species could be potentially used as a natural source of the anti-mosquito chemical.

Keywords: Cendana Papua, *Flindersia sp*, GCMS pyrolysis, mosquito repellent, phytochemistry

Abstrak

Penelitian ini bertujuan untuk mengidentifikasi komponen kimia dari kayu cendana Papua (*Flindersia sp*.) dan menganalisa potensi pemanfaatannya sebagai pengusir nyamuk. Metode eksperimen yang dilakukan adalah analisis fitokimia kualitatif, analisis GCMS Pirolisis, dan uji mortalitas nyamuk untuk menginvestigasi sifat anti-nyamuk dari jenis kayu ini berdasarkan SNI 06-3566-1994. Hasil analisis fitokimia menunjukkan adanya komponen fitokimia seperti alkaloid, saponin, flavonoid, terpenoid, glikosida dan fenolik. Analisis GCMS pirolisis mengindikasikan bahwa diantara 43 senyawa kimia, butylated hydroxyanisole (5,37%), carbamic acid (5,30%), 2,4 imidazolidinedione (2,13%) and hydrazin (1,23%) adalah senyawa yang berperan dalam mengusir nyamuk. Uji mortalitas nyamuk menggunakan sampel uji dengan komposisi serbuk cendana Papua dan tempurung kelapa sebesar 50:50 menunjukkan tingkat kematian nyamuk tertinggi (55,6%). Hal ini mengindikasikan bahwa jenis kayu ini berpotensi untuk dijadikan sebagai bahan kimia alami pengusir nyamuk.

Kata kunci: Cendana Papua, fitokimia, *Flindersia sp*, GCMS pirolisis, pengusir nyamuk

Introduction

Several tree species from natural forests have been investigated for their mosquitocidal activities. For instance, the wood bark of Gemor (*Alseodaphne sp.*) and shell of kemiri (*Aleurites molucca*) are used as natural mosquito coil conducted by Cahyana dan Rachmadi (2011). They reported that the plant extracts could repel and knock down mosquitoes.

Cendana Papua (*Flindersia sp*.) is hardwood species found in Papuan Forest that is still lesser known commercially due to lack of information about its essential characteristics and utilization (Sosef *et al*. 1998). In West Papua, this species is spread in the Regency of Wasior (Wondama Bay) and Ransiki District. The local community uses this wood as a primary material for making boats and rows and constructing houses. What is unique from *Flindersia* is that this wood releases a good smell when it is cut or burnt. For that reason, the local community calls it “Dupa wood” and also uses the wood to make incense. In addition, the local people frequently burn some pieces of the wood in their front- or back yard to repel mosquitoes and other insects. Based on local knowledge, it is suspected that Cendana Papua wood can potentially be a natural repellent and mosquito killer. This will be beneficial information, especially for the Papua region, which is a malaria-endemic area.

Malaria is a disease spread among humans by the bite of an infected Anopheles mosquito. Malaria is found mainly in tropical countries all around the world, including Indonesia. Up to date, the distribution of malaria is still high in Indonesia, precisely in Papua, West Papua, and East Nusa Tenggara (Kemkes 2018). In 2018, the total population in West Papua was around 850000 people. Of 1550 research subjects who performed blood tests, 1018 patients were positively affected by Malaria (Subekti *et al.* 2018).

Many efforts have been made to control the number of mosquitoes. The local government of West Papua, through Health Department, has carried out several programs, such as public education about preventing the development of mosquito larvae, fogging in communities' residences, and also free distribution of anti-mosquito larvae to the local community. However, the programs have not significantly resulted in the eradication of the mosquito population.

Moreover, the community uses commercial mosquito repellent; either in spray, lotion, or mosquito coil. Those repellents, however, contain synthetic chemicals that could be toxic and dangerous to humans, specifically children and pets. Therefore, it is necessary to substitute synthetic chemicals with plant-derivate chemicals, such as natural insecticide. The advantage of using phytochemicals is that the active compounds generated are biodegradable, and their use is safe for humans and pets. According to Robinson (1995), metabolic compounds contained in plants, such as alkaloid, tannin, and flavonoid, are usually responsible for biological properties used to control insects' development.

The analysis of chemical components of *Flindersia* wood has been carried out by Purnawati (2013). The result showed that the wood contains 45.5% α-selulosa, 24.7% lignin, and 0.50% ash. Extractives dissolved in cool water, hot water, and ethanol: benzene are respectively 4.82%, 7.17%, 4.30%. The high number of extractives indicates that this species is potential for natural chemical utilization. Therefore, further research on phytochemical screening and chemical compound identification is necessary to scientifically confirm the use of this species as an anti-mosquito agent by the local community. This research is expected to provide information about plant utilization based on local wisdom, specifically for *Flindersia* wood.

Materials and Methods

Methods and observation variables

The method used in this study was experimental with observation technique. The variables observed were phytochemical content (alkaloids, flavonoids, saponin, and tannins) of the wood, GCMS analysis of the wood, and the mortality rate of tested mosquitoes.

Research procedures

Phyto-chemical analysis of Flindersia sp sawdust

Sawdust from a tree 60 cm in diameter, with 80% heartwood and 20% sapwood (100 g), was extracted using the maceration method with 500 ml of methanol for one week. The methanol extract obtained was then evaporated using a rotary vacuum evaporator at 65 °C, to obtain dry extracts of *Flindersia sp*. wood. The crude extract was then used for the phytochemical test.

Alkaloid test is conducted as follows: The crude extract (0.2 g) added with 2 ml of 30% ammonia and 20 ml of chloroform was added and crushed in a mortar. The suspension was filtered, and the filtrate was collected in a sample bottle. Then to the filtrate was added 10 mL of 0.1 M HCl solution, shaken, and kept to form 2 layers. The acid layer was taken and put into 2 test tubes. The first test tube was added Dragendroff reagent, and the second tube was added Meyer reagent. The presence of alkaloid compounds was characterized by red or orange precipitation in the first tube and white precipitation in the second tube (Robinson 1995).

The following procedures are for flavonoid, saponin, and tannin compounds. The crude extract (0.2 g) was put into a beaker glass. Hot water (100 ml) was added to the beaker, and the suspension is boiled for 5 minutes. Afterward, 15 ml filtrate was collected and put in 3 test tubes with 5 ml filtrate in each tube. Magnesium powder was added to the first test tube, added with 1 mL of concentrated HCl, and added amyl-alcohol. The mixture was shaken and allowed to separate. Colors formed in the layer of amyl-alcohol indicate the presence of flavonoids. The second tube was shaken vertically for 15 seconds and allowed to stand for 10 minutes. The formation of stable foam indicates the presence of saponin. To the third tube is added 5 drops of 1 N NaOH solution and filtered. The filtrate was added with a solution of iron (III) chloride FeCl3 1%. The formation of dark blue or blackish green indicates a compound class of tannins (Robinson 1995).

Pyrolisis GC-MS Analysis

Wood powder is injected into the quartz chamber in a pyrolysis unit heated in an oxygen-free environment at 400 °C for 10 s. The mixture of these compounds is then incorporated into the Shimadzu GCMS type QP2010. GCMS uses helium as a gas carrier, a capillary column of Phase Rtx-5MS type (length 60 m and diameter 0.25 mm), pressure 100 Kpa, column temperature 50 °C, flow rate 0.85 ml min^{-1}. The result of the structure of a chemical component is determined by computer software based on the GCMS characterization graph (Purnawati 2013).

Anti-mosquito test

Preparing and rearing Anopheles sp. mosquitoes

The method of rearing mosquito is conducted based on the method applied by Cahyani and Rachmadi (2011) that have been slightly modified. At first, *Anopheles sp* mosquito larvae are collected from water reservoirs in several communities' residences in Manokwari. Then the mosquitoes are kept in a container of clean water. The container is placed into a rearing box sized (70x70x70) cm^3. During 1-2 days, larvae will turn into pupae. If mosquitoes have come out of the cocoon (3-5 days), honey is put in the box to feed the mosquitoes. Later, the mosquitoes transferred to the mosquito's test box (6 boxes of treatments) with 20 mosquitoes each box.

Production of mosquito coil

The 80-mesh sawdust of *Flindersia sp.* wood and coconut shells mixed with composition is displayed in Table 1. The coconut shell was used to lengthen the burning time of the coil. The different composition becomes treatment in producing mosquitoes.

Table 1 Types of coils and their composition of the sawdust of *Flindersia sp* wood and ground coconut shells

Types of coils	The ratio of wood and coconut shells, %
F100	100: 0
F75	75: 25
F50	50: 50
F25	25: 75
F0	0: 100

The mixture of each treatment was added with tapioca flour (5% of the total weight) and a small amount of water (as a mixer). The paste is then printed in a spiral mold of mosquito coil and pressed with a pressure of 25 kg/cm^2. The mosquito coil is then dried to reach air dry (approximately 12%).

The mortality rate of mosquito

The mosquito coil of each treatment is burned and set in the testing box contained 20 mosquitoes. Observation is carried out by calculating the time required to burn out the coil perfectly and calculating the dead mosquitoes every 30 minutes. The calculation of mortality rate of mosquitoes is the percentage of the amount the death mosquitoes and those which were tested.

Results and Discussions

Qualitative phytochemical analysis of *Flindersia* wood

The presence of phytochemicals enables the plant to have a defense mechanism and protection from various diseases, and they are helpful for healing and curing human diseases (Wadood *et al.* 2013). Phytochemical analysis was carried out to determine phytochemical compounds in the stem of *Flindersia* wood qualitatively. The analysis result is displayed in Table 2.

Table 2 Qualitative Phytochemical analysis of *Flindersia sp*. wood

Chemical compound	Indicator
Saponin	+
Tannin	-
Phenolic	+
Flavonoid	+
Alkaloid	+
Triterpenoid	+
Steroid	-
Glycoside	+

(+): presence, (-): absence

This study has revealed that almost all essential medical phytochemicals are present in *Flindersia* wood, such are alkaloid, flavonoid, glycoside, phenolic, saponin, and terpenoid. At the same time, tannin and steroids were not found in this wood species.

The result shows that this wood species contains almost similar phytochemical constituents to another hardwood species from Papua, *Tristania sp*. Arifudin *et al*. (2009) investigated phytochemical components contained in this wood species. The study found that the stem of *Tristania sp*. wood contains alkaloids, saponin, and flavonoids.

These metabolite groups in this wood species could play essential roles as insecticidal property, anti-bacterial, anti-viral, and antifungal activities. Donia *et al*. (2012) reported that phenolics and flavonoid contents in three plants from the Chenopodiaceae family were found to have insecticidal activity. Plants containing alkaloids are used in medicine to reduce headaches and fever. These are attributed to anti-bacterial and analgesic properties (Wadood *et al*. 2013). Terpenoids have various critical pharmacological activities, such as anti-viral, antimalarial, anti-bacterial, anti-inflammatory, anti-cancer, and inhibition to cholesterol synthesis. According to Tiwari *et al*. (2011), polyphenols have anti-microbial, antidiarrhoeal, and anthelmintic, almost play similar roles to alkaloids. Glycosides have the ability to inhibits the release of autocoids and prostaglandins.

The metabolites mentioned above generally have anti-bacterial, antimalarial, anti-microbial, and anti viral activities. Thus, it can be concluded that they have the ability to prevent and heal the diseases caused by bacteria, mosquitoes, microbes, and viruses.

In this study, of important phytochemicals components of medical plants, tannins and steroids were not found in the stem of *Flindersia sp*. wood., while steroid and triterpenoid have analgesic properties. Steroid and saponin play essential roles in central nervous system activities (Mir *et al*. 2013). In a previous study, tannin was also not found in *Tristania sp*. wood (Arifudin *et al.* 2009).

GC-MS Analysis

The analysis using GC-MS Pyrolisis detected the presence of 43 chemical compounds within *Flindersia* wood. Figure 1 shows several compounds with a concentration averagely higher than 5%, which are ethylic acid (9.02%), 4-allyl-2,6-dimetoxyphenol (6.62), levoglucosan (6.30%), acetone (5.39%), butylated hydroxyanisole (5.37%), and carbamic acid (5.30%).

The two last compounds may responsible for repelling or killing the Anopheles mosquito tested in this research. Necsi *et al*. (2010) revealed that butylated hydroxyanisole (BHA) was one compound that showed insecticidal activity against *Oryzaephilus surinamensis.* Carbamic acid in this wood could have anti-mosquito properties as Gupta (2014) found that carbamic acid has insecticide properties. DHSS (2015) reported a similar statement that the derivate of carbamic acid, called carbamic insecticide, is used to kill or control insects. Some carbamates also repel mosquitos.

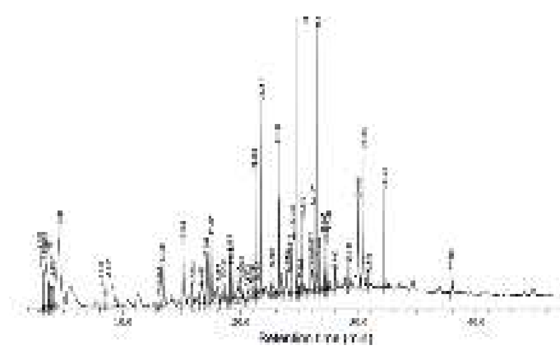

Figure 1 Result of GCMS Analysis

There are also several chemical compounds within *Flindersia* wood that are potentially used as an insecticide, such as 2,4 imidazolidinedione (2.13%) and hydrazine (1.23%). The first compound has been used as pest control and anti-microbial pesticide chemicals (EPA 2007). Thus, it could be a cause of mosquito mortality. Hydrazine which is contained within *Flindersia* wood is also suspected as an insecticide compound. Li *et al.* (2016) investigated gossypol, which also contains hydrazine. They found that the compound plays a role in anti-viral, insecticide, and fungicidal activities. These active compounds are possibly responsible for repelling insects, including mosquitoes, even cause mortality to the mosquito.

Some compounds can be extracted for food flavoring, pharmacy, and cosmetics, such as 4,5 epoxypentenal, guaiacol, p-ethyl guaiacol 4-allyl-2,6-dimethoxiphenol, vanillin dan syringaldehyde (Figure 1). Guaiacol is a naturally organic component generated from the pyrolysis of lignin. It can be used as a source of food flavoring, perfume, and cosmetics. Its derivates are potentially helpful for medicines as an expectorant, antiseptic and local anestacy (Guaiacol Safety Summary 2011). Syringaldehyde is a compound that naturally presents in Maple tree species used as a vanilla flavor in making wine (Arapitsas *et al.* 2004). According to Wong *et al.* (2010), syringaldehyde has been widely used in the food, drug, and cosmetic industries. As an aromatic compound, syringaldehyde can be produced either from derivate components of fossil oil or biomass.

In addition, *Flindersia* wood also contains a1-pentane-3-one, 1,5-diphenyl- around 1.61%. This compound has a simple aromatic ring to be potentially used as a source of perfume.

Anti-mosquito test: mortality of mosquito

Six types of Mosquito coil were made based on different compositions of Flindersia sp. wood sawdust and ground coconut shells. The types of coils are detailed in Table 1. The coils were addressed to mortality testing of mosquitoes. The coil was put into a testing box that contained reared mosquitoes. The coil was burnt for 30 minutes, and during the observation, the number of mosquitoes that died was counted. The mortality rate of mosquitoes tested is shown in Figure 2.

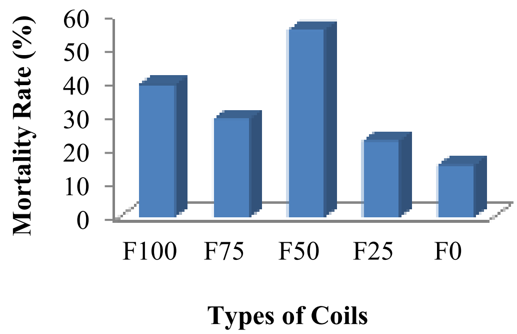

Figure 2 The effect of *Flindersia sp* sawdust on mosquito mortality rate

According to the Figure 2, the mortality of mosquito rate following the test of coils with sawdust compositions of 100%, 75%, 50%, 25% and 0% are 39.2%, 29.2%, 55.6%, 22.7%, dan 15.5%, respectively. This means that *Flindersia* wood contains active compounds that are toxic and then cause mortality to mosquitoes. On the other hand, the coil containing all coconut shells and no sawdust (F0) shows the lowest mosquito mortality rate.

This result is supported by the result of the phytochemical analysis (Table 2). The existence of mainly flavonoid and phenolic in Flindersia wood could play an essential role in insecticidal property.

Moreover, the result of GCMS analysis of this wood species also confirmed that butylated hydroxyanisole (5.37%), carbamic acid (5.30%), 2,4 imidazolidinedione (2.13%), and hydrazine (1.23%) have insecticide property that can repel mosquito.

This research found that the coil F50, with the composition of 50% Flindersia sawdust and 50% ground coconut shell, caused the highest mortality rate of the tested mosquito (Figure 2). This implied that this combination shows the best composition in producing coils that can kill a more significant number of mosquitoes. Flindersia sawdust contained several active compounds that have insecticide activity, as shown by the GCMS analysis result. At the same time, coconut shell only plays a role in prolonging the burning time of the coil. Jamilatun (2008) revealed that briquette made of coconut shells had a longer ignition time than that made of wood sawdust. Her research also found that coconut shell is responsible for the lower burning speed of briquette.

The result of this research agrees with the study conducted by Cahyana and Rachmadi (2011). The study results stated that the coil with a weight-based composition of 50:50 for Gemor wood bark (*Alseodaphne sp.*) and kemiri shell (*Aleurites molucca*) had the highest number of dead mosquito and also had longer and more stable burning capacity.

The local community has generally used Flindersia wood to repel mosquitoes by burning a piece of the wood in either their front or back yard. The smoke is also not toxic for human living. Thus, this wood species is recommended as a potential source of natural anti-mosquito that is not dangerous for man and pets.

Conclusions

Phytochemical analysis of *Flindersia sp.* showed the presence of alkaloids, saponin, flavonoid, terpenoid, glycoside, and phenolic.

GCMS analysis found 43 compounds contained in Flindersia wood. Butylated hydroxyanisole (5.37%), carbamic acid (5.30%), 2,4 imidazolidinedione (2.13%), and hydrazine (1.23%) may have insecticidal activity for the mosquito tested in this research.

According to the mosquito mortality test, the coil contained 50% Flindersia sawdust had the highest mortality rate of the tested mosquitoes (55.6%), while the coils without Flindersia sawdust showed the lowest mosquito mortality (15.5%). This wood species can be potentially used as a natural source of anti-mosquito.

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