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Antitermite Activity of *Eucalyptus pellita* Bark Extract

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ABSTRACT

The study uncovers the promising anti-termite properties of *Eucalyptus pellita* bark extract, a resource often discarded as industrial waste in the pulp and paper manufacturing industry. It investigates the anti-termite efficacy of various extracts from *E. pellita* bark and identifies the extract with the highest activity, followed by a comprehensive phytochemical analysis. Samples of *E. pellita* inner bark, outer bark, and mixed bark were ground into 40–60 mesh powder and subjected to extraction using soxhletation techniques with successive extraction in increasingly polar solvents (*n*-hexane, ethyl acetate, and methanol solvents). The anti-termite activity of these extracts was evaluated using no-choice bioassays to determine the termite antifeedant activity through the weight loss percentage of test samples and the mortality rate of *Coptotermes* sp. Furthermore, Py-GCMS analysis was conducted on extracts exhibiting the highest anti-termite activity to elucidate their chemical composition. The findings revealed that methanol extracts from all three parts of *E. pellita* bark exhibited the most potent anti-termite activity against *Coptotermes* sp., as evidenced by the highest mortality rate (100%) and cellulose paper weight loss of 9.57 ± 6.66 mg. These methanol extracts were predominantly composed of phenolic compounds, particularly condensed tannins, suggesting a potential breakthrough in termite control. The findings indicate that *E. pellita* bark may be a sustainable and eco-friendly alternative to chemical pesticides for termite control. Furthermore, its utilization may facilitate waste valorization in the pulp and paper industry, thereby supporting environmental sustainability and economic efficiency.

1. Introduction

Termites destroy plants and building structures worldwide, resulting in enormous economic losses. Subterranean termite species are significant pests in tropical and subtropical regions of Asia, causing damage worth hundreds of millions to billions of dollars (Kuswanto et al. 2015). These losses continue to increase annually due to using less durable wood species and poor construction (Debelo 2020). Some termite species also attack agricultural products, leading to production losses. The Perhutani Research and Development Center and the Faculty of Forestry of IPB also observed subterranean termite attacks on seedlings in two Forest Management Units, resulting in substantial damage and mortality among the infested seedlings (Puslitbang Perhutani

Fakultas Kehutanan IPB 2019). Synthetic termiticides are frequently used to control termite infestations. Still, synthetic termiticides pose environmental risks, such as the likelihood of contaminating groundwater, threats to endangered and vulnerable species, and potential impacts on endocrine systems (Oi 2022). Natural preservatives derived from wood, bark, leaves, seeds, and fruit may be an ecologically friendly alternative for termite prevention. Previous research has shown that many plant-derived compounds are effective against termites (Arinana et al. 2024; Prayogo et al. 2021a).

Eucalyptus pellita is a fast-growing tree native to northeastern Queensland, Australia. It has been widely planted in many sub-tropical and tropical regions, including Indonesia, as a primary fiber source for pulp and paper production (Hua et al. 2022). The location of HTI plantations, which are spread across various locations in Indonesia, means that logs of *E. pellita* must be delivered to pulp and paper mills, which are not located in as many plantations as those that grow *E. pellita*. Logs of *E. pellita* need to be debarked before shipping because the presence of bark will cause additional transportation weight and thus incur additional costs, resulting in *E. pellita* bark becoming waste. Paper quality generally disadvantages the bark due to its high levels of lignin and extractive compounds, which are removed before pulping. Bark also adds shipping costs and generally debarks from the logs before shipping (Neiva et al. 2020). This is because lignin is water-repellent and rigid and can also cause dark colors in the pulp, which requires a lot of bleaching agents (Lu et al. 2020).

The utilization of *E pellita* bark in Indonesia is still not maximally utilized. Widely used extracts, such as dyes, are usually derived from wood and are known to have antioxidant and antimalarial properties (Familasari et al. 2023; Pancapalaga et al. 2023; Prayogo 2023). Even the bark that is maximally utilized as a spice and medicine is only the well-known bark that is well known, such as *Cinnamomum burmanni* (Handayani et al. 2024). The bark has a protective function due to extractive compounds that help safeguard the inner wood and effectively cause mortality in adult insects (Kim et al. 2020). Extractive compounds extracted from the bark can be used as wood preservatives against insects and fungi (Nandika et al. 2023; Nkogo et al. 2022; Zalsabila et al. 2024). Previous studies have shown that extractive compounds from the mangium bark are effective against *Coptotermes gestroi* subterranean termites that consume wood impregnated with these extractives (Yingprasert et al. 2021). Bopenga et al. (2021) reported that extracts derived from the bark of *C. edulis* could thus serve as valuable components in developing natural bio-pesticides with potentially reduced environmental impact due to their organic origin. The anti-termite activity assessed by impregnating extracts onto the Whatman filter paper showed promising anti-termite properties, improving effectiveness as the solution concentration increased.

The bark generally comprises inner and outer bark with different extractive compounds. The inner bark, or phloem, is the tissue that serves as a channel to deliver food to all parts of the tree. The outer bark, called the periderm (and sometimes rhytidome), protects the tree from the impact and temperature extremes of weather and fire and against other external agents that can damage the plant's interior (Doyal 2004). Previous studies have shown that the outer bark of *E. globulus* contains several triterpenic acid compounds with lupane, ursane, and oleanane skeletons such as betulonic, 3-acetylbetulinic betulinic, 3-acetylursolic acid ursolic, oleanolic, and 3-acetyloleanolic. The inner skin contains sterols and triterpenoids with βsitosterol and β-amyrin as the main components. Small amounts of β-sitostanol, stigmasterol, 24-methylenecycloartanol, and triterpenoids α-amyrin and lupeol were also identified in the inner bark (Freire et al. 2002).

Until now, there has not been much research on the activity of compounds in the inner and outer bark of *E. pellita* against termite attack. Andika et al. (2023) reported that there are compounds of catechol, 3-methyl-catechol, resorcinol, 3,5-Dimethoxy-4-hydroxytoluene, and (E)-3,3'-Dimethoxy-4,4'-dihydroxystilbene which are derivatives of tannins and extractives that have potential as termiticides. Therefore, this study aims to test the anti-termite activity of extractive substances of *E. pellita* bark extracted with solvents with graded polarity. Analyze the content of chemical compounds contained in the most active *E. pellita* bark extract as an anti-crawler by Pyrolysis-Gas Chromatography-Mass Spectrometry (Py-GCMS). Therefore, this study aimed to explore the activity of *E. pellita* bark extracts against the subterranean *Coptotermes* sp. in a laboratory filter paper assay test and to identify the compounds contributing to any anti-termite activity.

2. Materials and Methods

2.1. Materials

The materials used in this study were *E. pellita* bark from PT. Arara Abadi, Sinarmas Forestry Riau comprising both inner and outer bark, *n*-hexane, ethyl acetate, methanol, Whatman cellulose paper, $NaClO_2$, glacial acetic acid, heat-resistant plastic, distilled water, acetone, NaOH, H_2SO_4 and subterranean termites *Coptotermes* sp. The tools used in this research are a Willey mill, soxhlet, rotary evaporator, vial bottle, IG3 funnel filter, oven, desiccator, erlenmeyer, water bath, magnetic stirrer, autoclave, Py-GCMS (Shimadzu, Japan), moisture analyzer (Shimadzu Type MOC63u, Japan), acrylic, and dental cement.

2.2. Material Preparation

E. pellita bark collected from PT. Arara Abadi Sinarmas Forestry Riau was removed from freshly felled trees from the plantation and separated into inner and outer bark along with whole bark. The bark was dried outdoors for three days until it reached air-dry moisture content before being ground to pass a 40–60 mesh screen. The moisture content of each bark fraction was determined using a Shimadzu MOC63u Moisture Analyzer Shimadzu, Kyoto, Japan (Zalsabila et al. 2024).

2.3. Extraction

The extraction involved multilevel extraction following previously described procedures (Tibuhwa 2012). Three grams of the whole, inner, or outer bark was wrapped in Whatman No. 1 filter paper and placed in a soxhlet containing 150 mL of reagent-grade *n*-hexane. Extraction began with an *n*-hexane solvent, and subsequent extractions were done using ethyl acetate and methanol solvents on the residue. The extraction process followed the succulent method. Each sample, consisting of three grams of *E. pellita* inner bark, outer bark, and mixed bark, sized 40–60 mesh and previously determined for moisture content, was wrapped in filter paper, resulting in five timbers. Each sample underwent soxhletation with 150 mL of *n*-hexane solvent until the filtrate in the flask turned colorless, indicating the complete dissolution of extractive substances in *n*-hexane. The collected filtrate was then removed from the base flask. Subsequently, soxhletation was conducted with ethyl acetate solvent until the filtrate in the flask became colorless, followed by further extraction using methanol solvent via soxhletation. The obtained filtrates were concentrated using a rotary evaporator equipped with a foaming sensor Buchi R 300 System ILAB Cibinong.

2.4. Anti-termite Activity Assay

The anti-termite activity of the *E. pellita* bark extracts was assessed against *Coptotermes* sp. in a no-choice laboratory test (Yamaguchi et al. 2002). Fifty mm diameter Whatman cellulose paper No. 1 filter paper was oven dried (40°C) and weighed before 200µL of 2.5% dilution of a given extract was added to the paper. The paper was redried and weighed to determine net mass. A total of 5 filter paper samples were prepared per extract. The Whatman cellulose paper was selected based on the limited extract available. This substrate provides a controlled and consistent environment, allowing for an accurate assessment of the extract's bioactivity despite the small sample size.

The termite exposures were performed in acrylic containers (80 mm diameter by 60 mm high), each with a 10 mm thick layer of dental cement at the bottom (**Fig. 1**). An 80 mm diameter plastic mesh was placed on the cement surface. A single treated or untreated filter paper disc was added to each chamber. Fifty *Coptotermes* sp. workers and five soldiers obtained from the termite laboratory at IPB University were placed into each unit. The chamber was placed on moist filter paper to help retain moisture, and the chambers were incubated in the dark at 28°C for 14 days. After the test, The number of live termites was counted to determine mortality, and the filter paper was removed, scraped clean of any termite frass, oven-dried at 40°C, and weighed to assess termite antifeedant activity through the percentage of cellulose paper mass loss. The test was considered successful if termite mortality in the controls did not exceed 55% (Harsono 2016). Extract efficacy was defined as the lowest concentration, producing 100% termite mortality.

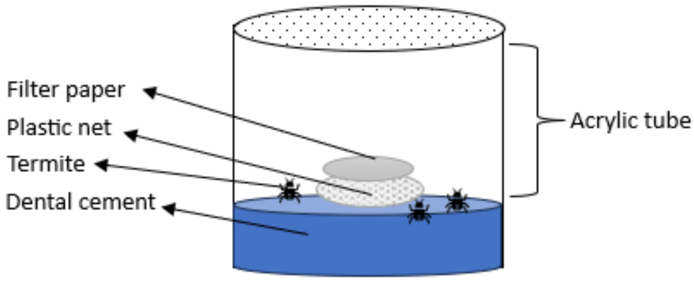

Fig.1. Illustration of the chamber used to assess the anti-termite activity of *E. pellita* bark extracts.

2.5. Chemical Compound Analysis of E. pellita Bark Extracts

The extracts analyzed were the extracts that caused the highest termite mortality from each part of the bark. The extracts were analyzed according to procedures described by Ismayati et al. (2016) utilizing pyrolysis-gas chromatography-mass spectrometry (PyGC/MS) on a Shimadzu GC/MS system QP-2020 NX equipped with a multi-shot pyrolyzer EGA/PY-3030D (Shimadzu, Kyoto, Japan). 200–500 µg of *E. pellita* bark extract was placed into SF PY1-EC50F eco-cups, then sealed with glass wool. These eco-cups underwent pyrolysis at 500°C for 0.1 minutes using helium gas on a SH-Rxi-5Sil MS column (30 m × 0.25 mm × 0.25 mm). Mass spectra were recorded at 70 eV with a pressure of 20.0 kPa (9.6 mL/min, column flow 0.60 mL/min). The temperature settings for py-GC/MS were programmed to start at 50°C for 1 minute, followed by an increase of 5°C/min until reaching 280°C, maintaining this temperature for 13 minutes. Identification of pyrolysis products was achieved by comparing retention times and mass spectra data with the NIST LIBRARY.

2.6. Data Analysis

The collected data were analyzed using SPSS software (version 25, New York, USA). This research used a factorial randomized complete block design (CRD). Two-way analysis of variance (ANOVA) was used to evaluate the variation in subterranean termite mortality and filter paper mass loss, considering bark extract and solvent type. Significance was determined at the 5% test level; means were further analyzed using the Duncan modified least significant difference test at $\alpha = 0.05$ Pallant (2020) to examine differences further.

3. Results and Discussion

3.1 The anti-termite activity

3.1.1 Mortality

Termite mortality is data from the level of termite death that occurs during the 14-day test period. Termite mortality data shows how much toxicity the extract substance has on the death of subterranean termites. The mortality value of *Coptotermes* sp. can be seen in **Table 1**. Termite mortality in the control treatment in this study reached 32.73%. The death of termites in control is thought to be due to the inability of termites to adjust to the new environment and the absence of other food sources besides the test paper fed (Arinana et al. 2024). In addition, dead termites will grow mold, which contains toxic substances that can kill insects so that other termites can die. Then, termites have the nature of necrophagy; termites will eat the carcasses of other termites and their weak or sick kind (Khan and Ahmad 2018).

Mortality of subterranean termites *Coptotermes* sp. in this study gave varied results. The analysis of variance showed that the interaction between the factors of the type of bark and the type of solvent affected the anti-termite activity of the extracts on termite mortality. Duncan's further test results showed that methanol-soluble outer bark, mixed bark, and inner bark extracts caused the highest termite mortality and were relatively the same. This treatment showed significantly different termite mortality when compared to treatments using other extracts. Mortality of the outer bark, mixed bark, and inner bark extracts dissolved in *n*-hexane and ethyl acetate showed results that were not significantly different from the mortality of *Coptotermes* sp. Mortality in the control without using solvents showed a value of 26.67 ± 2.10% and was significantly different from the negative control with *n*-hexane solvent, which was 32.73 ± 4.81% but not significantly different when compared to the negative control with methanol and ethyl acetate solvents. All control treatments differed considerably from all *E. pellita* bark extract termite mortality.

The variation in mortality rates for each solvent and each part of the bark type is thought to be due to differences in the resulting compounds drawn by each solvent. Houghton and Raman (1998) suggested that *n*-hexane can dissolve fats, waxes, fixed oils, and volatile compounds. Furthermore, the compounds dissolved by ethyl acetate are a group of alkaloids, aglycones, and glycosides. At the same time, methanol can attract compounds from the group of alkaloids, flavonoids, steroids, terpenoids, and tannins. Each extract from each solvent has been previously proven to attract bioactive compounds that can be used as pesticides to eradicate insects, including termites. Erliana et al. (2022), in a study of extracts from methanol solvents, produced high mortality against subterranean termites *C. curvignathus*. The same results were also from *n*-hexane and ethyl acetate extracts (Priadi et al. 2021).

The high mortality rate indicates that *E. pellita* extracts from various solvents contain bioactive compounds for termites. Bioactive compounds can disrupt the nervous system in termites. Damage caused by bioactive compounds can cause the termite's nervous system to malfunction, eventually leading to changes in body structure and the head that turns black until the termites finally die (Ahmed et al. 2016). According to Hassan et al. (2017), bioactive compounds can also kill protozoa and cellulose decomposers in the termite digestive system. So, if protozoa die from toxic extracts, the termite digestive system will be disrupted, causing death in termites. Radek et al. (2023) state that flagellate protozoa are found in the hindgut of termites in the form of *Retractinympha glossotermitis, Pseudotrichonympha* sp., and *Heliconympha* sp. The death of protozoa due to eating bait with bioactive compounds causes termites to be unable to digest cellulose properly, so the termites do not get the energy needed and will die.

3.1.2 Termite Antifeedant Activity

Observation of antifeedant activity indicated by the weight loss of cellulose paper is an alternative way to determine the anti-termite properties of an extract fed to termites apart from mortality. This test also looks at the attraction of termites to extracts applied to cellulose paper. The weight loss of cellulose paper from *E. pellita* bark extracts using various solvents fed to the subterranean termite *Coptotermes* sp. for 14 days is shown in **Table 1**.

Table 1. Effect of exposure to filter paper discs containing *E. pellita* bark extracts collected using three solvents on termite mortality and cellulose paper mass loss

Part of *E. pellita bark*	**Solvent**	**Mortality (%)**	**Mass loss (%)**
Control	none	26.7 ± 2.1^{a}	21.6 ± 0.6^{d}
Control	*n*-hexane	32.7 ± 4.8^{b}	24.9 ± 6.1^{d}
Control	Ethyl Acetate	30.9 ± 3.6^{ab}	19.8 ± 2.3^{d}
Control	Methanol	31.5 ± 3.8^{ab}	24.9 ± 2.4^{d}
Inner bark	*n*-hexane	78.2 ± 1.8^{c}	9.0 ± 3.0^{abc}
Inner bark	Ethyl Acetate	79.4 ± 3.8^{c}	$8.7 \pm 4.^{2abc}$
Inner bark	Methanol	100^{d}	6.5 ± 4.2^{ab}
Outer bark	*n*-hexane	83.6 ± 4.8^{c}	10.6 ± 0.9^{bc}
Outer bark	Ethyl Acetate	81.8 ± 3.6^{c}	11.4 ± 0.6^{bc}
Outer bark	Methanol	97.6 ± 4.2^{d}	6.2 ± 1.4^{ab}
Mixed bark	*n*-hexane	80.0 ± 4.8^{c}	13.2 ± 0.7^{c}
Mixed bark	Ethyl Acetate	83.6 ± 1.8^{c}	7.6 ± 2.1^{a}
Mixed bark	Methanol	100^{d}	4.7 ± 3.3^{a}

Notes: Values represent the mean of five replicates per treatment, while figures in parentheses represent one standard deviation. Values followed by the same letter (s) do not differ significantly by Duncan's modified least significant test at α=0.05.

The weight loss in **Table 1** showed diverse results from the type of *E. pellita* bark and the type of solvent used. The highest weight loss of cellulose paper was in the control with methanol solvent with a value of 24.94% (51.43 ± 4.05 mg). The analysis of variance showed that the interaction between the factors of the type of bark parts and the type of solvent affected the anti-termite activity of the extract on cellulose paper weight loss. Duncan's post-test results showed that the adverse control treatment in the form of solvent-treated paper and the control without treatment showed no significant difference at the 5% level. However, the weight loss of cellulose paper in all controls was significantly different compared to all *E. pellita* bark extract treatments with all solvents. The difference in the percentage of mass loss between the paper given the extract and the paper without extract (control) shows that all *E. pellita* bark extracts have antifeedant activity even though the percentage of paper weight loss between extracts is also different. The differences in solvent polarity have been shown to affect extract yield, type and composition of extractives, and their bioactivity (Andianto et al. 2024; Gioktavian et al. 2024).

Table 1 shows that the highest anti-termite activity based on the weight loss of cellulose paper was the methanol-soluble mixed bark extract of *E. pellita*. However, the weight loss of the cellulose paper was not significantly different compared to the treatments with methanol-soluble inner bark and outer bark extracts and ethyl acetate-soluble mixed bark. Cellulose paper treated with bark extracts with the highest weight loss was produced by cellulose paper treated with *n*-hexane soluble mixed bark extracts. Still, the weight loss was not significantly different from that of ethyl acetate and *n*-hexane soluble inner and outer bark extracts. The weight loss in *n*-hexane and ethyl acetate extracts indicates that non-polar and semi-polar solvents extract compounds with weaker termite-repellent properties than polar extracts in methanol solvent extracts. Previous research showed that methanol extracts derived from diverse plant sources have exhibited superior termiticidal efficacy. In particular, the methanol extract of *S. cumini* demonstrated a remarkable termiticidal potential, achieving a mortality rate of 74.67%, significantly higher than that observed with other extracts (Patel and Narasimhacharya 2017).

Furthermore, studies on fungus comb extracts from termites demonstrated that methanol extracts exhibited higher yields and bioactivity than hexane and ethyl acetate extracts. This finding supports the previous research that methanol is a more effective solvent for extracting termiticidal compounds (Witasari et al. 2022). Moreover, the presence of particular compounds in methanol extracts, including flavonoids and phenolic acids, contributes to their augmented bioactivity against termites. These compounds are frequently extracted in higher concentrations using methanol due to their polar nature, which facilitates the solvation of these bioactive molecules (Rachmayanti et al. 2022). In contrast, hexane extracts typically contain non-polar compounds, which may not exhibit the same level of biological activity against termites (Bayrak and Yanardağ 2021).

Table 1 shows that the weight loss of the test paper varied greatly depending on the bark and solvent type of the extract added to the test paper. The most weight loss of the test paper occurred in the control and the least in the methanol extract of mixed bark. This shows that the methanol extract given to the test paper contains a lot of toxins, so the more significant the refusal of termites to eat the test paper, the more the termites die. The decrease in termite consumption activity may be due to toxic compounds in *E. pellita* bark that slow down the feeding power of termites. The smaller the percentage reduction of the test paper, the higher the toxicity level of the extract. The results showed that the greater the mortality, the smaller the weight loss shown on cellulose paper. This aligns with research conducted by Afzal et al. (2019), which states that the relationship between weight loss and termite mortality is inversely proportional. However, these two parameters show the same phenomenon: the more significant the mortality of termites, the smaller the weight loss of cellulose paper, and vice versa; the smaller the mortality of termites, the greater the weight loss of cellulose paper fed to termites.

3.2 Phytochemistry of the E. pellita Bark Extracts

The phytochemical analysis of *E. pellita* bark extractives using Py-GCMS was carried out to determine the chemical compounds contained in *E. pellita* bark extracts with the highest mortality activity against *Coptotermes* sp. Py-GCMS can quickly analyze chemical compounds up to the macromolecular scale without needing preparation (Ismayati et al. 2016). The methanol extract showed the most active termite activity for each *E. pellita* bark extract from the mixed, outer, and inner bark. The results of Py-GCMS analysis showed that the methanol extract of the inner bark was detected to contain 45 types of compounds, the outer bark 35 types of compounds, and the mixed bark 39 types of compounds. Interestingly, the mortality data showed a reverse trend in which the outer bark extracts tended to result in higher termite mortality than the inner bark extracts. The outer bark is not solely a physical barrier but contains various chemical defenses. Previous studies showed conifers produce oleoresin, a mixture of terpenoids that can be toxic to insects. This oleoresin is primarily associated with the inner bark and phloem, where it can directly impact the feeding behavior of insects like bark beetles (Magerøy et al. 2020). Furthermore, the structural differences between outer and inner bark may influence the release and bioavailability of these compounds. Being more exposed to environmental factors, the outer bark may have evolved to produce higher concentrations of protective compounds in response to herbivory and other stressors (Ferrenberg and Mitton 2014).

Table 2 presents the ten types of compounds with the highest relative concentrations, indicating that the phenolic compound group dominates the extract. In addition, several fragmentations of carbohydrates were thought to be derived from hemicellulose, ester compound groups, waxes, short-chain organic acids (acetic acid), and long-chain organic acids (lipids). The program of the Py-GCMS analysis results is presented in **Fig 2**.

Table 2. Compounds of methanol extract from *E. pellita* bark are suspected to play a role in termite activity

Retention time	Pyrolysis products	Compound group	Relative abundance (%)		
			Mixed bark	Outer bark	Inner bark
2.56	Acetic acid	Organic acid	4.17	6.38	5.72
45.22	Bis(2-ethylhexyl) phthalate	Ester	4.52	6.09	2.33
44.94	Octan-2-yl palmitate	Ester	2.46	4.14	3.44
20.89	Pyrogallol	Phenolic	18.62	3.77	3.37
15.67	Catechol	Phenolic	16.54	6.28	17.87
12.15	Guaiacol	Phenolic	3.73	4.81	4.84
18.32	4-methyl-catechol	Phenolic	3.37	1.20	2.75
19.68	Syringol	Phenolic	3.06	5.83	4.80
21.22	Pyrogallol (overlapping)	Phenolic	2.31	-	3.42
17.28	3-methoxy-catechol	Phenolic	1.51	3.00	2.82
23.98	D-Allose	Carbohydrates	-	6.01	-
5.08	Furfural	Carbohydrates	1.51	4.81	3.46
45.09	2-methylhexacosane	Carbohydrates	0.93	5.56	2.26
34.23	methyl ester Hexadecanoic acid	Lipids	7.45	4.45	-
34.94	n-hexadecanoic acid	Lipids	5.05	-	-

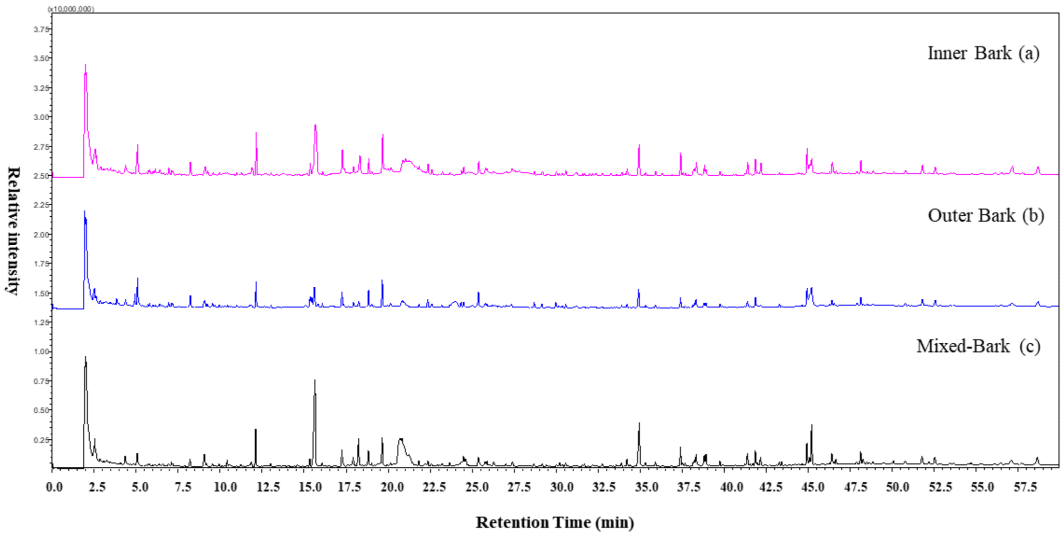

Fig. 2. Py-GCMS Pyrogram on methanol extracts of *Eucalyptus pellita* bark.

The presence of phenolic compounds in the methanol extract of *E. pellita* bark is thought to play an essential role in termite-repellent bioactivity. Previous research shows that *Acacia mangium* bark extracts hold significant potential as a natural material to enhance the durability of rubberwood (Yingprasert et al. 2021). The bark of *Acacia* spp. contains a lot of tannins, which are part of the phenolic compound in the form of condensed tannin, and polymer condensed tannin is potentially useful as a termite control agent (Ismayati et al. 2018). Phenolic compounds in plants function, especially in the bark, by contributing to cell wall formation, acting as a defense mechanism to regulate germination, and imparting unique aromas to plants (Raitanen et al. 2020). Py-GCMS pyrolysis products from the highest phenolic compound group are known to be tannins. Tannins are known to be found in bark (Ismayati et al. 2017). The fragmentation of tannins obtained were catechol, 4-methyl-catechol, 3-methoxy catechol, and pyrogallol. Pyrogallol and Catechol, 3-methoxy are pyrolysis products of epigallocatechin. The dimers of catechin and epigallocatechin indicate that the tannins in *E. pellita* bark are condensed tannins (Andika et al. 2023). Catechin has derivative products such as catechol and 4-methyl (Ismayati et al. 2017).

The relative abundance of tannins in methanol extracts of *E. pellita* bark was in line with the mortality of *Coptotermes* sp. The mortality of *Coptotermes* sp in inner and mixed bark methanol extracts was 100%, while that of outer bark averaged 97.58%. The relative abundance of catechol in *E. pellita* bark in inner and mixed bark was also higher than in outer bark. The relative abundance of catechol in *E. pellita* bark extracts in the outer bark and mixed bark is more than pyrogallol, indicating that tannins with catechin structures are more abundant when compared to epigallocatechin. Catechin is known to have higher termite-repellent properties than other condensed tannin dimers (Ismayati et al. 2018). Ismayati et al. (2017) stated that condensed tannins' different structures affect extractives' toxicity against termite infestation.

Furthermore, organic acids and lipids were detected in the methanol extract of *E. pellita* bark. The presence of organic acids (acetic acid) and lipids (Hexadecanoic acid, methyl ester, and n-hexadecanoic acid). Sudrajat et al. (2018) stated that hexadecanoic acid, methyl ester in *E. pellita* bark, is thought to play a role in the activity of subterranean termites *Coptotermes* sp. In addition, Prayogo et al. (2021b) stated that organic acids and lipids in *Melia azedarach* bark are the essential constituents that play a role in termite-repellent activity.

4. Conclusions

The bark extract of *E. pellita* has high anti-termite activity, with the highest anti-termite activity shown by methanol extracts in all three parts of the bark, indicated by the highest mortality value and lowest cellulose paper mass loss. Analysis of Py-GCMS showed that the methanol extract of *E. pellita* inner bark, outer bark, and mixed bark detected compounds dominated by phenolic groups, respectively. Tannin hydrolysis products found in *E. pellita* bark in the form of pyrogallol and 3-methoxy catechol are derivatives of epigallocatechin and catechol, and 4-methyl catechol, which are derivatives of catechin. The relative abundance of Py-GCMS shows that the levels of pyrogallol and catechol are almost the same in the inner bark. Still, the abundance of catechol in the outer and mixed bark exceeds pyrogallol. This research is limited to no-choice bioassay testing. Further research is needed to explore the mechanism of action of these compounds and their effectiveness under field conditions. Future studies could also investigate the environmental impact of using *E. pellita* bark extracts in large-scale termite control.

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