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Sandoricum koetjape Merr. (Meliaceae), also known as wild mangosteen, is a kind of tree that produces fruits, and various parts of the tree have medicinal properties. Antitermite and antifungal activities of *S. koetjape* heartwood extract were tested to develop effective and environmentally friendly agents to control termites and wood-rotting fungi. The heartwood mill was extracted with acetone and then methanol giving their extracts, which were successively fractionated using *n*-hexane, ethyl acetate, and water to afford their six soluble fractions. The yield of the extracts and fractions suggested that the extractives of *S. koetjape* tend to be non-polar. The antitermite test against a subterranean termite, *Coptotermes formosanus* revealed that the acetone extract and its fractions had the strong antifeedant activity classified into III-IV of the antifeedant class, and that the methanol extract and its fractions had lower antitermite activity belonging to I-II of the antifeedant class. The antifungal test indicated that against *Trametes versicolor* the *n*-hexane fraction of the methanol extract had the strongest activity belonging to the strong category according to the antifungal activity values, while against *Fomitopsis palustris* all the extracts and fractions had the weak category.
Keywords: *Sandoricum koetjape*, *Coptotermes formosanus*, antifeedant activity, *Trametes versicolor*

Sandoricum koetjape 心材抽出物のイエシロアリ *Coptotermes formosanus* 及び木材腐朽菌に対する効果

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Sandoricum koetjape Merr.（センダン科）は，野生マンゴスチンとしても知られていて，果樹の一種であり，そして，その樹木のさまざまな部位が薬効を持つ。*S. koetjape* 心材抽出物の抗蟻性と抗真菌活性を，効果的かつ環境に優しいシロアリ防除剤と防腐剤を開発するために試験した。心材粉末はアセトン次いでメタノールで抽出し，得られた両抽出物は，逐次，*n*-ヘキサン，酢酸エチル及び水を用いてそれぞれの可溶部に分画し，全部で六画分を得た。これらの抽出物と画分の収率から， *S. koetjape* の心材抽出物には非極性成分が多い傾向にあることが示唆された。イエシロアリ *Coptotermes formosanus* に対する抗蟻性試験では，アセトン抽出物及びそれからの三画分が強い摂食阻害活性を有し，摂食阻害レベル III～IV であったこと，そして，メタノール抽出物及びそれからの三画分は，低い摂食阻害活性を有し，摂食阻害レベル I～II であることが明らかになった。抗真菌活性試験では，*Trametes versicolor* に対しては，メタノール抽出物からの *n*-ヘキサン画分が最も強い活性を有し，これは強い活性レベルであり，一方，*Fomitopsis palustris* に対しては，これらの抽出物と画分のすべてが弱い活性レベルであることがわかった。
キーワード：*Sandoricum koetjape*, *Coptotermes formosanus*，摂食阻害活性，*Trametes versicolor*

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1. INTRODUCTION

Biological deterioration is a main factor that degrades the durability of wooden houses, including fungi and termites. Among all factors leading to biodegradation, termites are most damaging to wooden structures worldwide. Biodegradation of wood caused by termites is recognized as one of the most serious problem for wood utilization. A Formosan subterranean termite, *Coptotermes formosanus* Shiraki, is a native of East Asia and the most devastating termite pests. For controlling termites, synthetic termiticides have been used for a long time. To avoid environmental pollution and health problems caused by the use of traditional wood preservatives or synthetic pesticides, there is increasing interest in naturally occurring toxicants from plants[1]. Natural products are a promising source of compounds exhibiting pesticidal activities [2,3,4].

On the other hand, wood-rotting fungi lead to great economic losses of lignocellulosic materials. Fungi play various important roles in the forest ecosystem, including ectomycorrhizal fungi, arbuscular mycorrhizal fungi, saprotrophic or wood-decomposing fungi, plant pathogenic fungi, and bioremedial fungi[5]. The infection by fungi reduces the durability and quality of the wood. Nature has produced a large variety of plants with an array of survival and defensive chemical strategies including insecticidal and fungicidal components.

S. koetjape, which produces wild mangosteen fruit, is one of wood species that explore potentially its bioactive compounds as termiticide and fungicide. This wood is light and durable, and so it is usually used for component parts of house such as battens or rafters[6], and widely used as building materials, boats, carts, and crates[7]. Various parts of the tree have medicinal properties[8]. *S. koetjape* bark containing sandoricum acid is traditionally used to cure a skin disease called *Tinea corporis* or ringworm. The fresh leaves are applied to treat diarrhea, and decoction or infusion of the leaves is used for baths to reduce fever. The roots are used as a tonic and in Malayan medicine as a preventive after childbirth and as a general tonic. Moreover, aqueous extract of the bark has been consumed as a tonic after childbirth. In Indonesia, the bark decoction has been used to treat leucorrhea and colic[8].

In this study, therefore, the efficacy of *S. koetjape* heartwood extracts against a subterranean termite of *C. formosanus* and against wood rotting fungi of *Trametes versicolor* and *Fomitopsis palustris* were investigated.

2. MATERIALS AND METHODS

2.1. Wood species

Sandoricum koetjape Merr. wood was collected from Soppeng Regency, South Sulawesi Province, Indonesia, with 60 years of age.

2.2 Extraction and fractionation

Heartwood mill (40-60 mesh) of *S. koetjape* was prepared and extracted according to the procedure reported previously[9] with slight modification. In this study, the milled wood that was used instead of small wood-pieces was first extracted with acetone, and then the residue was extracted again with methanol until the extract solution became colorless. These acetone and methanol extracts were then successively fractionated into *n*-hexane, ethyl acetate, and water to give their soluble fractions.

2.3 Termite

Coptotermes formosanus Shiraki used was collected in black pine forest on the coast of Kochi Prefecture, Japan and bred in Forestry and Forest Products Research Institute, Tsukuba, Japan.

2.4 Fungal strains

The fungal strains used were a white-rot fungus (*Trametes versicolor*, NBRC 4937) and a brown-rot fungus (*Fomitopsis palustris*, NBRC 30339) that were purchased from Biological Resource Center, National Institute of Technology and Evaluation, Tokyo, Japan.

2.5 Termite bioassay

No-choice and two-choice tests were employed to assess the termiticidal activity of *V. cofassus* heartwood[10]. The weight loss of the paper discs was used to determine termiticidal properties of the extracts that were obtained by the following equations[10]: in the no-choice bioassay, the absolute coefficient of antifeedancy (A) = [(KK – EE)/(KK + EE)] x 100 (%); while in the two-choice bioassay, the relative coefficient of antifeedancy (R) = [(K – E)/(K + E)] x 100 (%); where KK (K) and EE (E) are the weight losses of the control and treated paper discs, respectively. The total coefficient of antifeedancy (T) is equal to A plus R. All the extracts tested were classified into the following classes according to their T values[10]; feeding preference ($T < 0$), class I ($0 \leq T < 50$), class II ($50 \leq T < 100$), class III ($100 \leq T < 150$), class IV ($150 \leq T < 200$), and 200 for complete antifeedant.

2.6 Fungal bioassay

Fungal bioassay was conducted using the potato dextrose agar (PDA) medium containing 50 and 100 ppm of samples (the extracts and the fractions) in a Petri dish[11]. Triplicate media for each amount of the samples were prepared. The PDA powder in distilled water (39g/L) was autoclaved, and to this warm medium (40-50°C) was added a solution of each of the samples in methanol, and the whole was shaken. Three parts from the resulting mixture were transferred equally into three Petri dishes, respectively, and the mycelium disk was placed at the center of the medium. PDA plates containing methanol without the samples were used as a control. The media were incubated at 23°C. When the mycelium of fungi on the control medium reached the edge of the Petri dish, the antifungal activity (AFA) was calculated as follows: antifungal index (%) = (1－Da/Db) x 100, where Da: diameter of mycelium colony growth with the samples (cm), Db: diameter of mycelium colony growth in the control (cm)[12]. Based on the AFA value, the activity of each fraction was then classified into the following category levels[12]: AFA ≥ 75% (very strong), 75% ≤ AFA < 50% (strong), 50% ≤ AFA < 25% (moderate), 25% ≤ AFA < 0% (weak), and 0% (not active).

2.7 Statistical analyses

The SPSS software (IBM SPSS Statistics Version 21) was utilized as a statistical tool. As a result of multiple analysis of variation (ANOVA) test, the type of all the samples (the extracts and fractions) were evaluated their antitermite and antifungal activities. For post hoc analysis, Scheffe's test was used to compare the values at a level of significance of $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Extract yield

Extraction of *S. koetjape* heartwood and subsequent fractionation of the extracts showed that the acetone extract had higher percentage (76.5%) than the methanol extract (2.3%). The *n*-hexane fraction of the acetone extract had the highest percentage (45.9%) compared to the other fractions, namely the ethyl acetate (28.7%) and aqueous fractions (2.9%). Moreover, the ethyl acetate fraction of the methanol extract had high percentage (34.8%) compared to the *n*-hexane (24.1%) and aqueous fractions (25.3%). These data suggested that the *S. koetjape* heartwood extractives tend to be non-polar.

The extractives comprise both inorganic and organic components. The inorganic components were measured as ash and its content did not exceed 1% of the dry wood weight. The organic components are an extraordinarily large number of individual compounds of both lipophilic and hydrophilic type, and their contents are usually less than 10%, but it can vary from traces up to 40% of the dry wood weight[13].

As the case of more than 10% (dry weight) of the content of wood extractives, the genus *Larix* is known as having an exceptionally high content of extractable components in the heartwood, usually within the wide range of 5–30%[14]. The wood extractive content is classified as high, if it is more than 4%[15]. High extractive content of the *S. koetjape* heartwood in this study was probably due to the nature of *S. koetjape*.

3.2 Antitermite activity

3.2.1 Weight loss

Weight loss of the no-choice bioassay displayed on Figure 1 shows that the *n*-hexane fraction of the methanol extract had the lowest weight loss compared to the other fractions. Furthermore, weight loss of the two-choice bioassay presented in Figure 2 indicated that the *n*-hexane fractions of the acetone and methanol extracts had the lowest weight loss.

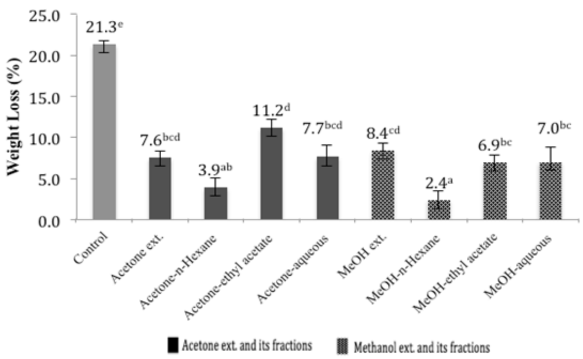

Figure 1. Weight loss of paper discs in the no-choice bioassay.

Notes: Different letters at the top of the bars indicate the significant difference of the weight loss at the level of $P < 0.05$ according to the Scheffe's test.
a: The lowest weight loss and significantly different from d and e.
ab: The weight loss not significantly different from bc or abc.
bc: The weight loss not significantly different from abc.
d: The weight loss significantly different from a and e, but not significantly different from bcd or cd.
bcd: The weight loss not significantly different from abc or cd.
cd: The weight loss not significantly different from abc or bc.
e: The highest weight loss and significantly different from a, c, and d.

The data suggested that there were significant differences in protection of the extracts and their fractions against *C. formosanus*. The lower weight loss represents their protection ability against the termites. No-choice bioassay method is for testing termiticidal activity, while two-choice bioassay method is for testing antifeedant activity[10].

These results confirmed that *S. koetjape* heartwood extracts and their fractions had termiticidal activity, which indicated that they were able to deliver reliable protection against *C. formosanus*. Furthermore, the two-choice bioassay as shown on Figure 2 indicated that these extracts and fractions had antifeedant activity.

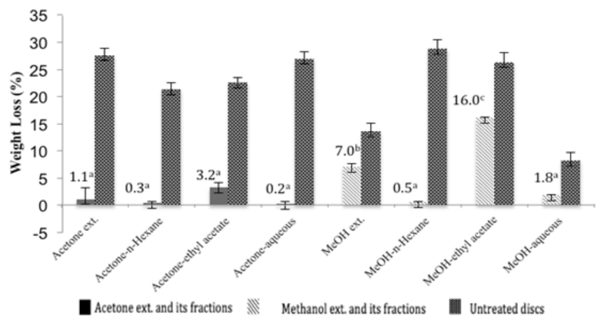

Figure 2. Weight loss of paper discs in the two-choice bioassay.

Notes: Different letters at the top of the bars indicate the significant difference of the weight loss at the level of $P < 0.05$ according to the Scheffe's test.
a: The lowest weight loss and significantly different from b and c
b: The weight loss significantly different from a and c
c: The highest weight loss and significantly different from a and b

3.2.2 Antifeedant activity

The no-choice bioassay indicated that the *n*-hexane fraction of the methanol extract had the highest antitermite activity and was classified into class II of antifeedancy classification. Otherwise, the two-choice bioassay indicated that the *n*-hexane fraction from both the acetone and methanol extracts had the highest antifeedant activity and was also classified into class II of antifeedancy classification. Extractives present in these extract could be toxic or unfavorable for the termites.

Studies about antitermite active compounds of *S. koetjape* are still less until now. It was reported that sandoricin and 6-hydroxysandoricin from *S. koetjape* seeds had antifeedant activity against armyworm *Spodoptera frugiperda* (J.E. Smith) and European corn borer *Ostrina nubilalis* (Hubner) larvae[16].

On the other hand, the ethyl acetate extract of teak wood was responsible for the greater resistance of the heartwood against termites[17]. Some flavonoids, such as quercetin and taxifolin might be useful for termite control agents, because they are abundant in plants[10]. The resistance to termite attack is due to the presence of some active components in wood as part of their natural defense, such as flavonoids that possess natural repellent having both toxicity and antioxidant properties[18]. On the other hand, a study conducted previously indicated that water-soluble extract could contain sugar[19], and another study stated that the residue fraction could contain substances, such as vanillin and something else that are digestible and preferable by termite[20].

The total coefficient of antifeedancy (T) was determined as the sum of the absolute coefficient of antifeedancy of the no-choice bioassay (A) and the relative coefficient of antifeedancy of the two-choice

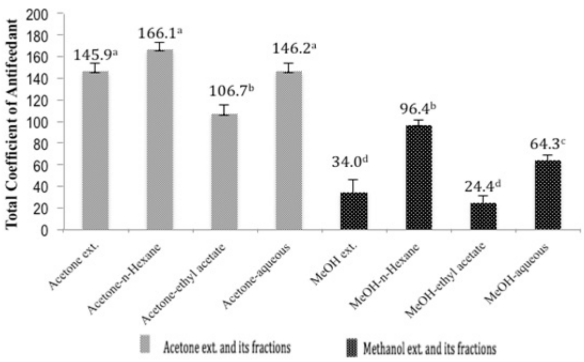

Figure 3. Total coefficient of antifeedancy of *S. koetjape* extracts and their fractions.

Notes: Different letters at the top of the bars indicate the significant difference of the total coefficient of antifeedancy at the level of $P < 0.05$ according to the Scheffe's test.
a: The highest total coefficient of antifeedancy and significantly different from b, c, and d.
b: The total coefficient of antifeedancy significantly different from a, c, and d.
c: The total coefficient of antifeedancy significantly different from a, b, and d.
d: The lowest total coefficient of antifeedancy and significantly different from a, b, and c.

bioassay (R). Figure 3 displays the total coefficient of antifeedancy (T) of *S. koetjape* extracts and their fractions, which were classified into the five classes according to their T values[10]. The acetone extract and its fractions had high antifeedancy including to class III-IV of antifeedancy classification. On the other hand, the methanol extract and its fractions had lower antifeedancy that were classified into class I-II of antifeedancy classification.

3.3 Antifungal activity

The antifungal test against *T. versicolor* (Figure 4) showed that the *n*-hexane fractions from the methanol extracts of *S. koetjape* at 50 and 100 ppm had the highest activity, while the *n*-hexane fraction of the acetone extract and the methanol extract had the lower activity.

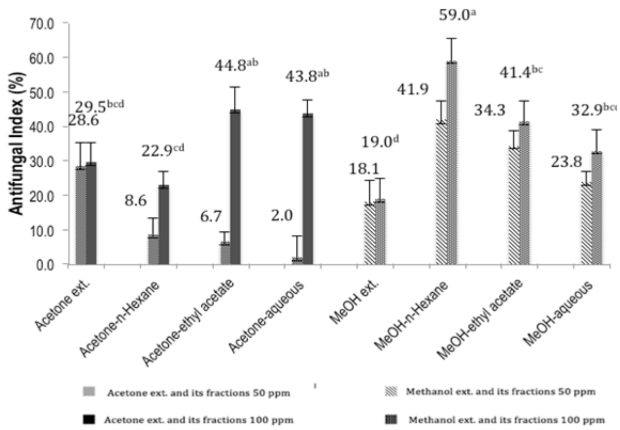

Figure 4. Antifungal Index of *S. koetjape* extracts and their fractions against a white-rot fungus, *Trametes versicolor.*

Notes: Different letters at the top of the bars indicate the significant difference of the antifungal index at the level of $P < 0.05$ according to the Scheffe's test.
a: The highest antifungal activity but not significantly different from ab.
ab: The antifungal activity not significantly different from a, bc or bcd.
bc: The antifungal activity not significantly different from ab, cd or bcd.
cd: The antifungal activity not significantly different from bc or bcd.
bcd: Not significantly different from ab, bc, or cd.

According to the antifungal classification[12], the *n*-hexane fraction of the methanol extract was classified into strong activity, and most of the other extracts and fractions showed moderate activity at 100 ppm, except for the *n*-hexane fraction of the acetone extract and the methanol extract that belonged to the weak category.

The order of antifungal index of the extracts and fractions against *T. versicolor* at 100 ppm (Figure 4) was *n*-hexane fraction of methanol extract > ethyl acetate fraction of acetone extract > aqueous fraction of acetone extract > ethyl acetate fraction of methanol extract > aqueous fraction of methanol extract > acetone extract > *n*-hexane fraction of acetone extract > methanol extract.

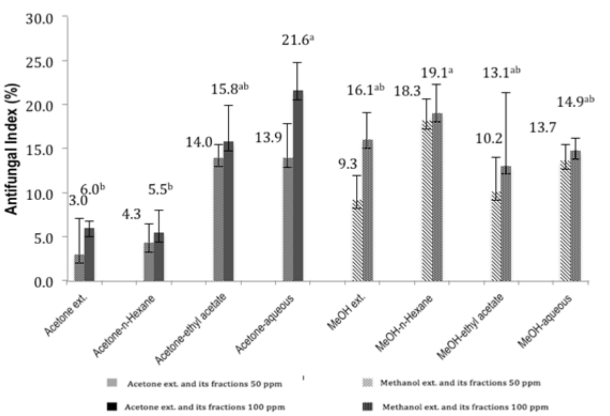

Figure 5. Antifungal Index of *S. koetjape* extracts and their fractions against a brawn-rot fungus, *Fomitopsis palustris*.

Notes: Different letters at the top of the bars indicate the significant difference of the antifungal index at the level of $P < 0.05$ according to the Scheffe's test.
a: The highest antifungal activity and significantly different from b, but not significantly different from ab.
b: The antifungal activity significantly different from a, but not significantly different from ab.
ab: The antifungal activity not significantly different from a or b.

On the other hand, the antifungal test against *F. palustris* (Figure 5) indicated that the *n*-hexane fraction of the methanol extract and the aqueous fraction of the acetone extract at 100 ppm had the highest activity, and that the acetone extract at 50 ppm and the ethyl acetate fraction of the methanol extract revealed the lowest activity. According to the antifungal classification[12], The aqueous fraction of the acetone extract and all the extracts and fractions were classified into weak activity.

The order of antifungal index of the extracts and fractions against *F. palustris* at 100 ppm (Figure 5) were aqueous fraction of acetone extract > *n*-hexane fraction of methanol extract > methanol extract > ethyl acetate fraction of acetone extract > aqueous fraction of methanol extract > ethyl acetate fraction of methanol extract > acetone extract > *n*-hexane fraction of acetone extract.

Brown-rot fungi degrade lignocellulosic materials via modification of the plant cell wall, induced both by non-enzymatic systems and by hydrolytic enzymes[21]. Each of wood species has different physical and chemical properties, affecting their susceptibility to the fungal infections[22]. The strong activity of the extracts means that they strongly inhibits the fungal growth.

The antifungal active compound from *S. koetjape* has not been obtained until now. However, there are several reports about antifungal active compounds from the other plants[23,24]. Extracts from cinnamon leaves have proved highly effective against wood decay fungi and termites and can potentially be developed into excellent organic preservatives. The antifungal effectiveness of cinnamaldehyde and eugenol against a white-rot fungus, *Lenzites betulina* and a brown-rot fungus, *Laetiporus sulphureus* was evaluated[24]. Cinnamaldehyde, a major constituent of cinnamon essential oils, occurs naturally in the bark and leaves of cinnamon trees of the genus *Cinnamomum*. The antifungal activity relating to chemical structures also tested and found that cinnamaldehyde, α-methyl cinnamaldehyde, (*E*)-2-methylcinnamic acid, eugenol, and isoeugenol were very effective against the fungi tested, and that the presence of aldehyde and/or carboxyl groups, a conjugated double bond, and the length of CH chain had an influence on the activity[24].

Liriodenine, an alkaloid, which was isolated and identified from the *n*-hexane fraction of *Michelia* *formosana* could effectively inhibit the growth of wood-rotting fungi[5]. Then, eugenol has been demonstrated as an excellent fungicide against wood decay fungi[11]. Moreover, the high antifungal activity of mimosa and quebracho extracts was believed to be related to their high tannin contents[22]. T-muurolol and α-cadinol possess antifungal activities against a broad spectrum of tested plant pathogenic fungi and could be used as potential antifungal agents[24].

4. CONCLUSIONS

The present study revealed the antitermitic and antifungal activities of *S. koetjape* heartwood extracts. The yield of the acetone extract, methanol extract, and their fractions suggested that the heartwood extractives tend to be non-polar. The antitermite test against *C. formosanus* indicated that the acetone extract and its fractions had the significant antifeedant activity and classified into III-IV of antifeedant class. In addition, the antifungal test against *T. versicolor* showed that the *n*-hexane fraction of the methanol extract had the strongest activity, while the extracts and the other fractions almost belonged to moderate-weak activity. On the contrary, against *F. palustris* all the extracts and fractions had weak antifungal activity.

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