

Journal of Tropical Biodiversity and Biotechnology Volume 10, Issue 01 (2025): jtbb11823 DOI: 10.22146/jtbb.11823

Research Article

Antifeedant Activity of Limonoids from the Seeds of *Lansium domesticum* Corr. Against Subterranean Termite *Coptotermes curvignathus*

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Keywords:

Coptotermes curvignathus Dukunolide Lansium domesticum Limonoid Meliaceae Submitted: 19 January 2024 Accepted: 27 June 2024 Published: 10 January 2025 Editors: Ardaning Nuriliani Tanti Agustina

ABSTRACT

Lansium domesticum is one of Meliaceae plants produces limonoids with various biological activities, except for anti termites. Seven limonoids, dukunolides A-D (DA-DD), F (DF), and langsatides A-B (LA and LB), each previously isolated from the seeds of *L. domesticum* and prepared at 5 %, together with methanol root extract (MRE) 5 % were evaluated for insecticidal activity against *Coptotermes curvignathus*. Fifty workers and five soldiers of *C. curvignathus* were tested in a No-Choice Test to determine which limonoid was the most active. Dukunolides A-D, F, langsatides A-B, and MRE showed weaker antifeedant activity than the regent 50sc (positive standart, 8.04 %), except for dukunolide B (DB) that was stronger antifeedant activity, with a 7.28 % paper weight loss and 33.3 % mortality against *C. curvignathus*. Conclusion, this study showed limonoid compounds that were isolated from the seeds of *L. domesticum* could be developed for antitermite drugs.

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How to cite:

Rudiyansyah et al., 2025. Antifeedant Activity of Limonoids from the Seeds of *Lansium domesticum* Corr. Against Subterranean Termite *Coptotermes curvignathus*. *Journal of Tropical Biodiversity and Biotechnology*, 10(1), jtbb11823. doi: 10.22146/jtbb.11823

INTRODUCTION

Limonoids are derived from tetracyclic triterpenoids by several oxidative changes, obtained mainly as secondary metabolites in plants of Rutaceae and Meliaceae (Roy & Saraf 2006; Nebo et al. 2015; Lin et al. 2022). These compounds exhibited various bioactivities such as antibacterial, antifungal, antiviral, anticancer, antimalarial, and insecticidal. Limonoids such as azadirachtins A and B are major natural products isolated from the seeds of *Azadirachta indica* (Meliaceae) showed insecticidal properties and exhibited very low toxicity to mammals and birds (McKenzie et al. 2010). Many insecticidal activities of limonoids from several genera of Meliaceae plants have been reported (Roy & Saraf 2006; Happi et al. 2018; Sun et al. 2018; Lin et al. 2022). However, scarce information about antifeedants has been reported, particularly antitermite activity.

One of Meliaceae plants, *Lansium domesticum*, grows mostly in Southeast Asia, has a fruit used as a popular dessert. However, the peels are believe to be toxic to domestic animals. In the Philippines and Borneo, indigenous people control mosquitoes by burning leaves and bark of *L. domesticum* (Monzon et al. 1994; Leaman et al. 2015). The seeds were also reported to contain antimalarial constituents, namely domesticulides A-E (Saewan et al. 2006). Moreover, kokosanolide A, isolated from the seeds of *L. domesticum* cv Kokossan, showed an antifeedant activity against instar larvae of *Epilachna vigintioctopunctata* (Mayanti et al. 2011).

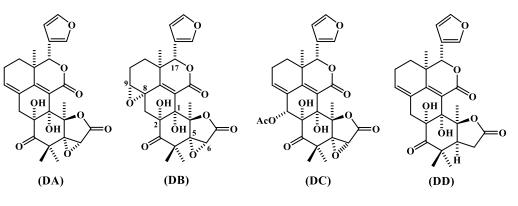
A scientific effort has been made to design drugs from medicinal plants; in contrast, investigation to develop insecticides has played a minor role (Nakayama & Osbrink 2010). A vast number of plant extracts has been explored and screened to search the insecticidal compounds, particularly for anti -termites; however, only a few phytochemical compounds have been used commercially to control them (Bourminta et al. 2013). The methanol extract of the heartwood of *Calophyllum inophyllum* revealed termiticidal activities (Kadir et al. 2015). Further, chemical constituents such as 5-phenyl-2-(1propynyl)-thiophene and 1-phenylhepta-1,3,5-tryne that were isolated from stems of *Coreopsis lanceolata* showed antitermitic activity against the subterranean termite *C. curvignathus* (Pardede et al. 2018). In addition, leaf extracts from clove (*Syzigium aromaticum*) and cajuput (*Melaleuca cajuputi*) have been tested for paper weight loss and mortality against subterranean termite *C. curvignathus* (Indrayani et al. 2016).

Coptotermes genus contains approximately 28 species and they are known to cause serious problem for environment (Su & Scheffrahn 1998). Coptotermes sp. is the main species of subterranean termites and the most aggressive one. In continuation of our concern for insecticidal constituents, seven limonoids, dukunolides A-D (DA-DD) and F (DF), and langsatides A-B (LA-LB), which have been previously obtained from the seeds of *L. domesticum* Corr. (Rudiyansyah et al. 2018) and the methanol root extract (MRE) of *L. domesticum* Corr. were evaluated for anti-termite against *C. curvignathus*.

MATERIALS AND METHODS

Samples and Filter Papers Preparation

The root of *L. domesticum* Corr. was obtained from Pontianak, West Kalimantan, Indonesia. It was powdered (300 g) and macerated with methanol for 3 x 24 hours to give a dried methanol root extract (MRE) (12 g) by a rotary evaporator at 40 °C. Each limonoid, dukunolides A-D (DA-DD), F (DF), langsatides A-B (LA-LB) (Figure 1), and methanol root extract (MRE) was dissolved in chloroform and methanol, respectively to prepare a 5% (w v⁻¹) solution. Those limonoids were isolated from the seeds of *L. domesticum* (Rudiyansyah et al. 2018). Filter papers (Whatman No. 1, 50 mm diameter) were weighed and soaked with 5% (w v⁻¹) of each tested sample and MRE for approximately an hour, air-dried at room temperature for a day, and reweighed before the test (Indrayani & Alkhadi 2021; Oramahi et al. 2023). The control papers were treated with distilled water (negative) and regent 50sc (positive).



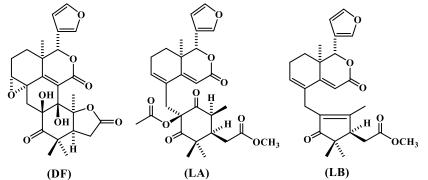


Figure 1. Dukunolides A-D (DA-DD), F (DF), and langsatides A-B (LA-LB) isolated from the seeds of *L. domesticum* Corr. (Rudiyansyah et al. 2018).

Termites Preparation

Subterranean termites *C. curvignathus* were collected from the local rubber forest, Pontianak, West Kalimantan, Indonesia. Termites and rubber wood (*Hevea brasiliensis*) were conditioned inside the perforated plastic containers at 28-30 °C (70-80 % humidity) for a month. Termites with good health conditions were selected (five soldiers and fifty workers), relocated into a new container, and they were not feeding for 24 hours before the bioassay.

Termite Bioassay

A no-choice test with some modification was carried out for anti-termite activity against *C. curvignathus* (Ohmura et al. 2000; Güzel et al. 2017; Quiroz et al. 2017; Liu et al. 2019). The tests were conducted in plastic cups (bottom diameter 6 cm, height 6 cm). Each cup was filled with sterilised sea sand (50 mesh, height \pm 1 cm) on the bottom and moistened with 3 mL of distilled water. Each treated paper was placed on the plastic plate (40 mm diameter) and subsequently it was set on top of sea sand, then 55 termites were introduced. All treatment units were stored at 27-28 °C and 70-82 % humidity in the dark room for 3 weeks. Each treatment was maintained for five replicates, including the control papers (filter papers soaked in distilled water and regent 50sc, respectively). The weight loss of filter papers and termite mortality were measured when the test period ended.

RESULTS AND DISCUSSION

Paper Weight Loss and Termite Mortality

Paper weight loss is significant to investigate in order to show the preference for termite to eat bait. All tested samples showed paper weight losses from 7.28 to 11.6 %, much smaller than the negative control paper, which was 80.85 % (Figure 2). Dukunolide B (DB) gave a paper weight loss of 7.28 % and other limonoids DA, DC-DD, and LA-LB including dukunolide F (DF) showed weight loss of 11.6 %. Furthermore, dukunolide B also exhibited a bit stronger paper weight loss than the positive control, that was 8.04 %. Although the paper weight loss values differed among all tested papers, however these were not significant difference except for the negative control paper. These paper weight losses were presumably because each limonoid consists of the furan ring and epoxide groups (Bentley et al. 1988; Roy & Saraf 2006; Nebo et al. 2015; Happi et al. 2018).

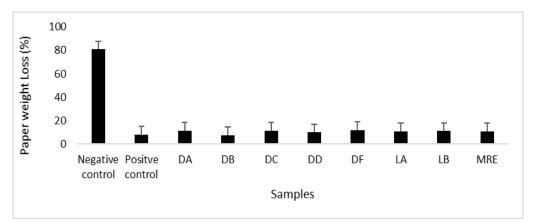


Figure 2. Paper weight loss of limonoids and MRE subjected for 3 weeks to C. curvignathus.

The efficacy of other limonoids could also be seen from the weight loss percentages of tested papers after being subjected to *C. curvignathus*. Except for DB, all treated papers displayed similar weight loss, 11.34, 11.29, and 11.66 % for DA, DC, and DF, respectively (Figure 2). Even though the epoxide position is different between structures DA and DC at C5/C6 and DF at C8/C9, the attachment of a furan ring at C-17 is the same (Matos et al. 2014; Shi et al. 2020). Further, limonoids DD, LA, and LB that only had a furan ring at C-17 resembled paper weight loss activity to limonoids contained both the furan ring and epoxide groups. As a positive control in this study, synthetic anti-termite (regent 50sc) was used with a concentration of 5 %. The value of paper weight loss in the positive control was smaller then other treatments, which was 8.04 % except for DB with a weight loss of 7.28 %. It can be said that DB is potential as an environmentally friendly termite repellent.

Additionally, anti-termite activity between MRE and all tested limonoids was not distinguishable, indicating that it contained limonoids or similar types of compounds. Based on these data, all limonoids DA-DD, DF, and LA-LB together with MRE had activity against subterranean termite *C. curvignathus*. Some literatures about structure-activity studies have discussed that limonoids with a furan ring and epoxides on their structures are associated with antifeedant activity (Bentley et al. 1988; Roy & Saraf 2006; Nebo et al. 2015). For example, the limonin which is the main chemical constituent from *Citrus* plants and contains a furan ring at C-17 and the epoxide on the structure exhibited antifeedant activity against beetle larvae (Bentley et al. 1988).

Similar to paper weight loss activity, the percentages of termite mortality for all tested samples were indistinguishable, around 30.00 %, except DB (21.09 %) (Figure 3). These values were two-fold higher than the negative control paper (14.54 %) and three-fold lower than the positive control paper (100 %). Compound DB was less toxic than regent 50sc, indicating that it should pose less risk to human and environmental health.

All limonoids DA-DD, DF, and LA-LB have the same sidechain furan ring attached at C-17. Again, this finding supported literature that a furan ring at C-17 in limonoid structures has an important role in antifeedant activity (Bentley et al. 1988; Roy & Saraf, 2006; Matos et al. 2014; Nebo et al. 2015; Happi et al. 2018; Shi et al. 2020) including against subterranean termite *C. curvignathus*. In other words, in terms of mortality, there is no significant difference in the position of the epoxide, sidechain ring, and other groups in limonoid structures.

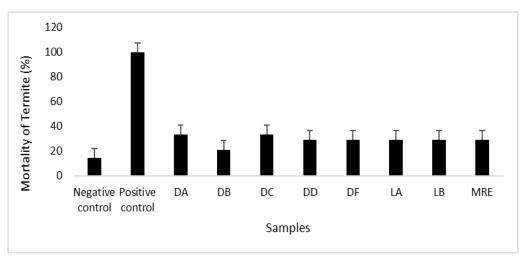


Figure 3. Percentages mortality of limonoids and MRE against termite of C. curvignathus.

CONCLUSIONS

These results proved that the tested limonoids from the seeds and methanol root extract of *L. domesticum* Corr. could be a potential eco-friendly pest management and probably developed into antitermite drugs.

AUTHOR CONTRIBUTION

R. and Y.I. designed, analysed the data, and wrote a manuscript, A.H.A. and A.S. supervised all stages of research, and E.P.M. run and collected the data.

ACKNOWLEDGMENTS

This work was financially supported by Universitas Tanjungpura (Grant No. SP DIPA-042.01.2.400955/2019).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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