**Original Article**

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# Onoceranoid Triterpene from the Stembark of *Lansium domesticum* Corr. Cv. pisitan and Their Larvicidal Activity

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## Abstract

*Lansium domesticum* Corr. is a plant of the Meliaceae family known to produce metabolites that are beneficial for insect control and medicine. This plant can be found in almost all provinces in Indonesia. *L. domesticum* Corr consists of three varieties, namely duku, kokosan and pisitan. *L. domesticum* Corr. duku and kokosan varieties are known to have various secondary metabolite compounds including the onoceranoid triterpene and showed various biological activities, namely antifeedant, larvicide, antimalarial, and cytotoxic. The aim of this study was to obtain isolated compound and determined its chemical structures of triterpenoid compound from ethyl acetate extract of pisitan stem bark (*L. domesticum* Corr. Cv. pisitan) and its larvicide activity against *Aedes aegypti* larvae. The isolation process begins with maceration of pisitan bark with methanol then continued with partitioning of the methanol extract with *n*-hexane and ethyl acetate. The ethyl acetate extract was then separated and purified using column chromatography techniques. The pure isolate compound **1** was further characterized using mass spectroscopy, infrared spectroscopy, $^{1}$H-NMR, $^{13}$C-NMR, and 2D NMR. The chemical structure of the isolated compound was determined based on spectroscopic data and by comparison with previously obtained spectral data. The isolated compound **1** was identified as lansiolic acid, which is first time isolated from this variety. The larvicidal activity of the compound against *A. aegypti* larvae showed a percentage of larval death of 90% after 48 hours of observation at a test solution concentration of 1%.

**Keywords**: triterpenoid, onoceranoid, *Lansium domesticum* Corr. Cv. pisitan, lansiolic acid, larvicidal activity.

## INTRODUCTION

Meliaceae family is a rich source of valuable secondary metabolites compound including triterpenoid [1]. *L. domesticum* is a plant of the Meliaceae family known to produce compounds that are beneficial for insect control and medicinal materials. This plant is widely spread in Indonesia island. *L. domesticum* is a unique plant that are divided into three different varieties such as, duku, kokosan, dan pisitan [2].

The compounds that were successfully isolated from *L. domesticum* Corr. is grouped into several types, namely, the onoceranoid triterpene, lansioside, domesticulide, dukunolide, cycloartanoid, and kokosanolide [3]. The onoceranoid triterpene has been isolated from various parts of the *L. domesticum* plant, including lansic acid from the skin of fruit [4], lansiolic acid from leaves [5], and six onoceranoid triterpene compounds from bark named iso-onoceratriene, onoceradienedione, 3-keto-22-hydroxyonoceradiene, lansiolic acid, lansiolic acid A which showed antifeedant activity against *Sitophilus oryzae* [6]. Lansiolate acid was successfully re-isolated from stembark with two other onoceranoid triterpene compounds, namely 3β-hydroxyonocera-8(26),14-dien-21-one and 21α-hydroxyonocera 8(26), 14-dien-3-one [7]. In the kokosan variety, the onoceranoid triterpene compound group was successfully isolated from the kokosan bark named 8,14-secogammacera-7,14-dien-3,21-dione and 8,14-secogammacera-7-en-14-hydroxy-3,21-dione which showed

antifeedant activity [8,9]. The onoceranoid triterpene group is a group of compounds found in almost all parts of the *L. domesticum* plant, duku and kokosan varieties.

Only small publications regarding the chemical content of the pisitan variety make it necessary to explore the potential of onoceranoid triterpene compounds from this variety. In addition, the larvicidal activity of *L. domesticum* Corr. Cv. pisitan is an interesting thing because generally *L. domesticum* is a plant known to have antifeedant activity. In this research we isolated and determine the chemical structure of lansiolic acid (**1**) (Figure 1) from the stembark of *L. domesticum* Corr. Cv. pisitan, together with the larvicidal activity against *A. aegypti* larvae.

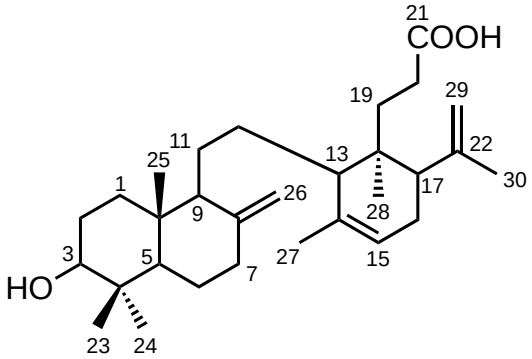


Figure 1. Chemical structure of compound **1**.

## EXPERIMENTAL SECTION

### Material and Instrumental

The Shimadzu 8400 FTIR (in KBr) was used for FTIR spectrum The JEOL-type NMR spectrometer was utilized for the otaining of one and two-dimensional NMR (500 MHz for $^{1}H$ and 125 MHz for $^{13}C$) using additional of tetramethylsilane (TMS, internal standard). The Waters Q-TOF type Xevo mass spectrometer was used for the determination of molecular weight together with molecular formula. Column chromatography for purifications using Silica gel (size 70-230 mesh and size 230-400 Mesh, Merck for normal phase and size 100-200 mesh for reverse phase ODS) as stationary phase. Silica gel $GF_{254}$ and RP-18 $F_{254}$ plates (Merck, 0.25 mm) were employed for the TLC and visualization was conducted by spraying the plates with a 10% $H_2SO_4$ solution in ethanol, followed by heating.

### Plant Material

The sample used in this study was pisitan stembark (*L. domesticum* Corr. Cv. pisitan) obtained from the Bogor area, West Java, Indonesia. This material was determined in the Plant Anatomy Laboratory in Department of Biology, Universitas Padjadjaran by Joko Kusmono with specimen number 10188.

### Methods

### Extraction and Isolation

The powder of dried stembark of *L. domesticum* Corr. cv. pisitan (2.5 kg) was extracted using maceration technique with methanol for six days (6 x 10 L). After solvent evaporation under vacuum, the 26 g of methanol extract was obtained. The thick brown methanol extract was dissolved in water then partitioned with *n*-hexane and ethyl acetate to give 2.4 g of *n*-hexane extract and 18.9 g of ethyl acetate extract.

Ethyl acetate extract was continued for separation using vacuum liquid chromatography technique with silica gel 60 using combination of stepwise elution of *n*-hexane:ethyl acetate (100:0-0:100, 10% increasing polarity) continued by stepwise elution of ethyl acetate:methanol (100:0-0:100, 10% increasing polarity) to obtain 5 fractions (A-E). The fractions C (5.6 g) was separated again using vacuum liquid chromatography using same solvent system as previous to produce six subfraction (C1-C6). The subfraction C1b (1.9 g) containing the main component was separated by open column chromatography with silica gel 60 with a gradient solvent (stepwise *n*-hexane:ethyl acetate, 10:0-1:1, with increasing polarity 5%) then the fraction containing the target compound (C1b5, 121 mg) was purified by open column chromatography with silica gel 60 isocratic with a chloroform:methanol (9.5: 0.5) to give 15 mg of pure compound **1**. The isolated compound

was characterized by UV, IR, NMR and MS spectroscopy methods for the determination of its chemical structure.

### Determination of Larvicidal Activity

The test solution consisted of a 1% ethyl acetate fraction solution, a 1% pure isolate solution, and a control solution without treatment was prepared. Each solution was added with 10 *A. aegypti* mosquito larvae. Observations were made after 24 hours and 48 hours, and the number of dead larvae was counted to determine the percentage of mortality [10].

## RESULTS AND DISCUSSION

Several stages of column chromatography were utilized to purify the isolated compound **1** (Figure 1). Its chemical structure was meticulously analyzed based on spectroscopic data, including FTIR, MS, and NMR.

Compound **1** was obtained as a white amorphous powder. The chemical formula of compound **1** was identified as $C_{30}H_{48}O_3$, with an ion peak in TOFMS at *m/z* 457.3626 $[M+H]^+$ (calculated as 457.3682 for $C_{30}H_{49}O_3^+$). Functional group which existed in compound **1** according to FTIR were O-H (3468 $cm^{-1}$), C-H $sp^2$ (2935 $cm^{-1}$), *gem*-dimethyl (1442 and 1381 $cm^{-1}$), C-O (1075 $cm^{-1}$), C=O carboxyl (1710 cm-1), and C=C (1660 $cm^{-1}$). Observation of $^1$H NMR of spectrum demonstrated six tertiary methyl groups ($\delta_H$ 0.67, 0.76, 0.90, 0.81, 1.77, and 1.73, each 3H), one oxymethine at $\delta_H$ 3.26 (1H, dd, *J*=4.8, 11.4 Hz), one $sp^2$ methine at $\delta_H$ 5.36 (1H, brs) and two $sp^2$ methylene at $\delta_H$ 4.5 & 4.83 (each 1H, s) and 4.70 & 4.82 (each 1H,s). The numbers of methyl groups indicated the presence of onoceranoid triterpene skeleton [9]. The $^{13}$C NMR spectrum (Table 1) showed 30 signals. The DEPT experiment identified these as six methyls, eleven methylenes (including two $sp^2$ methylenes at $\delta_C$ 106.9 and 114), six methines (including one $sp^2$ methine at $\delta_C$ 121.8 and one oxymethine at $\delta_C$ 79.4), and seven quaternary carbons (including three $sp^2$ quaternary carbon at $\delta_C$ 148.4, 136.1, and 147.8 and one carboxylic acid group at $\delta_C$ 178.0). The alkene and carboxylic acid functional groups accounted for four out of seven degrees of unsaturation, with the residual three degrees were attributed to a tricyclic onoceranoid triterpene skeleton. The positioning of functional groups and the main skeleton of compound **1** were confirmed through the HMBC and $^1$H-$^1$H COSY (Figure 2). The HMBC each methyl group with adjacent carbons confirmed the onoceranoid-type structure. The correlations of H-23,24 to C-3,4, and 5, and of H-2 to C-3, determine the position of hydroxyl group at C-3. The position of each double bond was confirmed based on HMBC. Crosspeak correlation of H-26 to C-7, C-8, and C-9 determined the position of double bond at C-8/C-26. Double bond at C-14/C-15 was observed based on correlation of H-27 to C-13, C-14, and C-15. This resulted correlation was the key correlation to determined that compound **1** was onoceranoid triterpene or 8,14-seco-gammacerane structure [8]. Another double bond unambiguously appearsed at C-22/C-29 based on correlation of H-29 to C-17, C-22, and C-30. Carboxylic acid group observed at C-21 resulted from the correlation of H-19 and H-20 to C-21. The formation of double bond at C-22/C-29 and carboxylic acid group at C-21 revealed that there is a cleavage of ring D in onoceranoid structure at C-21/C-22. The $^1$H-$^1$H COSY experiment also supported the HMBC correlation. $^1$H-$^1$H COSY correlation of H-1/H-2/H-3 proofed the presence of ring A, whether the correlations of H-5/H-6/H-7 proofed the presences of ring B. Ring C can be observed based on correlations of H15/H-16/H-17 together with the presence of double bond at C-14/C-15. The NMR data of compound **1** was compared with lansiolic acid obtained from the fruit peel of *L. domesticum* [5] and found to be identical including its all stereocenter in compound **1**. Thus, compound **1** was identified for the first time from *L. domesticum* variety of pisitan. From the view of chemotaxonomic, the discovery of compound **1** in pisitan variety confirms the relationship between varieties within the species *L. domesticum*. Based on all spectroscopic evidence, compound **1** determined as lansiolic acid.

**Table 1**. $^{1}$H NMR (500 MHz) and $^{13}$C NMR (125 MHz) data of compound **1** in $CDCl_3$.

| No. | $^{13}$C NMR δc (mult.) | $^{1}$H NMR $\delta_H$ (Integral, mult., *J*=Hz) |
|---|---|---|
| 1 | 37.1 | 1.81 (2H, m) |
| 2 | 29.6 | 1.24 (2H, m) |
| 3 | 79.4 | 3.26 (1H, dd, *J*=3 and 9 Hz) |
| 4 | 39.2 | - |
| 5 | 54.6 | 1.08 (1H, m) |
| 6 | 27.9 | 1.39 (2H, m) |
| 7 | 38.2 | 1.98 & 2,37 (each 1H, m) |
| 8 | 148.4 | - |
| 9 | 58.5 | 1.56 (1H, m) |
| 10 | 38.7 | - |
| 11 | 26.1 | 1.09 (2H, m) |
| 12 | 24.1 | 1.36 (2H, m) |
| 13 | 48.4 | 1.85 (1H, m) |
| 14 | 136.1 | - |
| 15 | 121.8 | 5.36 (1H, brs) |
| 16 | 27.4 | 1.62 (2H, m) |
| 17 | 49.2 | 2.19 (1H, m) |
| 18 | 39.5 | - |
| 19 | 32.6 | 1.64 (2H, m) |
| 20 | 28.9 | 2.43 (2H, m) |
| 21 | 178.0 | - |
| 22 | 147.8 | - |
| 23 | 28.4 | 0.98 (3H, s) |
| 24 | 15.6 | 0.76 (3H, s) |
| 25 | 14.7 | 0.67 (3H, s) |
| 26 | 106.9 | 4.50 & 4.83 (each 1H, s) |
| 27 | 23.0 | 1.73 (3H, s) |
| 28 | 16.4 | 0.81 (3H, s) |
| 29 | 114 | 4.70 & 4.82 (each 1H, s) |
| 30 | 23.1 | 1.77 (3H, s) |

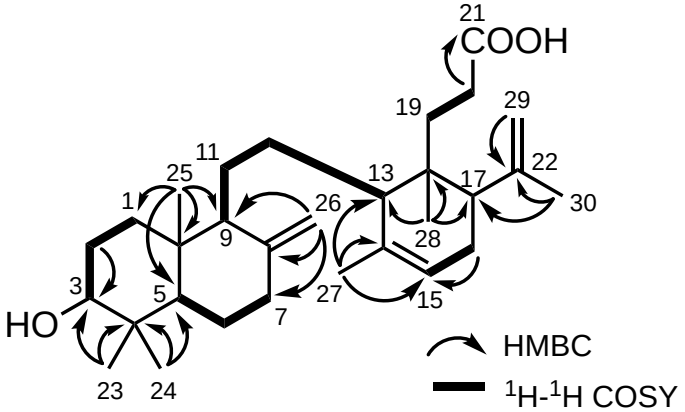


Figure 2. Selected HMBC and $^{1}$H-$^{1}$H COSY of compound **1**.

Ethyl acetate extract together with compound **1** (1% concentrations) was tested for its larvicidal activity against *A. aegypti* instar III larvae. The data (Table 2) showed that lansiolic acid (**1**) has the ability as a larvicidal agent. Larvae that are treated with 1% of compound **1** experience death of up to 90%. The similar resulted also observed in ethyl acetate extract. Therefore, the ethyl acetate extract and compound **1** has high potency as larvicidal against *A. aegypti* larvae.

Table 2. The number of living *A. aegypti* after treatment for 1 and 2 days.

| Sample | Number of *A.aegypti* larvae | *A.aegypti* living Day-1 | *A.aegypti* living Day-2 |
|---|---|---|---|
| Negative control | 10 | 10 | 10 |
| Ethyl acetate extract | 10 | 4 | 1 |
| Compound **1** | 10 | 3 | 1 |

## CONCLUSION

An onoceranoid triterpene compound was isolated from the stembark of *A. cucullata* for the first time and identified as *L. domesticum* Corr. cv. *pisitan*. The chemical structure of compound **1** was identified with FTIR, MS, NMR (1 and 2D), as well as comparison with previous literature. The larvicidal activity of compound **1** against *A. aegypti* larvae showed that compound **1** has mortality rate up to 90% in 48 hours experiments.

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## REFERENCES

1) Oyedeji-Amusa, M. O.; Sadgrove, N. J.; Van Wyk, B. E. The ethnobotany and chemistry of South African Meliaceae: A Review. *Plants* **2021**, *10*, 1796. DOI: https://doi.org/10.3390/plants10091796
2) Mayanti, T.; Sinaga, S. E.; Supratman, U. Phytochemistry and biological activity of *Lansium domesticum* Corr. species: A review. *J. Pharm. Pharmacol.* **2022**, *74*(*11*), 1568-1587. DOI: https://doi.org/10.1093/jpp/rgac057
3) Lubis, M. F.; Hasibuan, P. A.; Syahputra, H.; Astyka, R. A Review on phytochemicals and pharmacological activities as ethnomedicinal uses of duku (*Lansium domesticum* Corr.). *OAMJMS* **2022,** *10*(*F*), 57-65. DOI: https://doi.org/10.3889/oamjms.2022.8394
4) Dong, S. H.; Zhang, C. R.; Dong, L.; Wu, Y.; Yue, J. M. Onoceranoid-type triterpenoids from *Lansium domesticum*. *J. Nat. Prod.* **2011**, *74*(*5*), 1042-1048. DOI: https://doi.org/10.1021/np100943x
5) Nishizawa, M.; Nishide, H.; Kosela, S.; Hayashi, Y. Structure of lansiosides: biologically active new triterpene glycosides from *Lansium domesticum*. *J. Org. Chem.* **1983**, *48*(*24*), 4462-4466. DOI: https://doi.org/10.1021/jo00172a004
6) Omar, S.; Marcotte, M.; Fields, P.; Sanchez, P. E.; Poveda, L.; Mata, R.; Jimenez, A.; Durst, T.; Zhang, J.; MacKinnon, S.; Leaman, D. Antifeedant activities of terpenoids isolated from tropical Rutales. *J. Stored Prod. Res.* **2007**, *43*(*1*), 92-96. DOI: https://doi.org/10.1016/j.jspr.2005.11.005
7) Matsumoto, T.; Kitagawa, T.; Teo, S.; Anai, Y.; Ikeda, R.; Imahori, D.; Ahmad, H. S. B.; Watanabe, T. Structures and antimutagenic effects of onoceranoid-type triterpenoids from the leaves of *Lansium domesticum*. *J. Nat. Prod.* **2018**, *81*(*10*), 2187-2194. DOI: https://doi.org/10.1021/acs.jnatprod.8b00341
8) Mayanti, T.; Azahra, S. A.; Syafriadi, T. P.; Nurlelasari; Maharani, R.; Julaeha, E.; Supratman, U.; Sinaga, S. E.; Ekawardhani, S.; Fajriah, S. Onoceranoid triterpenes of *Lansium domesticum* Corr. cv. kokossan and their cytotoxicity against MCF-7 breast cancer cells. *Trends in Sciences* **2025**, *22*(*1*), 9000. DOI: https://doi.org/10.48048/tis.2025.9000

9) Mayanti, T.; Zulfikar; Fawziah, S.; Naini, A. A.; Maharani, R.; Farabi, K.; Nurlelasari; Yusuf, M.; Harneti, D.; Kurnia, D.; Supratman, U. New triterpenoids from *Lansium domesticum* Corr. Cv *kokossan* and their cytotoxic activity. *Molecules* **2023**, *28*(*5*), 2144. DOI: https://doi.org/10.3390/molecules28052144
10) Muniandy, P. D.; Riswari, S. F.; Ruchiatan, K. Larvicidal activity of *Citrus aurantifolia* decoction against *Aedes aegypti* larvae. *Althea Medical Journal* **2020**, *7*(*1*), 35-39. DOI: https://doi.org/10.15850/amj.v7n1.1814