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Dukunolide D from the Root of *Lansium domesticum* Corr. cv Kokosan

Angela Suryana, Andi Hairil Alimuddin, Rudiyansyah Rudiyansyah*

Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Tanjungpura, Jl. Prof. Dr. H. Hadari Nawawi, Pontianak, 78124, Indonesia.
*Corresponding author: rudiyansyah@chemistry.untan.ac.id

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Abstract: *Lansium domesticum* has three varieties and one of the varieties is kokosan. These plants contain limonoids as the main constituents. Phytochemical investigation of L. domesticum cv kokosan has identified onoceranoids-type triterpenoids and tetranortriterpenoids from the seeds, fruit peels, and bark except for the root. This research described dukunolide D **(1**) that has been obtained from the methanol extract of the root of kokosan. Methanol root extract was fractioned by n-hexane and dichloromethane (DCM). All fractions including the crude extract were screened for phytochemical test. The DCM fraction was chromatographed in order to obtain compound **1**. DART-HRMS of compound **1** showed a positive ion peak at m/z 469.1861 [M+H]+ (calculated 469.1862 corresponding for $C_{26}H_{29}O_8$). The structure of 1 was fully determined by 1D (1H-NMR, 13C-NMR, and DEPT-135) and 2D NMR (COSY, HSQC, HMBC, and NOESY), and by comparison with data from literature.

Keywords: dukunolide D, kokosan, *Lansium domesticum*, limonoid, Meliaceae

***Abstrak*:** *Tumbuhan Lansium domesticum memiliki tiga varietas dan salah satu nya adalah kokosan. Tumbuhan genus ini mengandung limonoid sebagai komponen utama. Penelitian kandungan fitokimia pada L. domesticum cv kokosan telah ditemukan adanya senyawa triterpenoid tipe onoceranoid dan tetranortriterpenoid dari biji, kulit buah, dan kulit batang kecuali akar. Penelitian ini menjelaskan struktur senyawa dukunolide D (**1**) yang telah diisolasi dari ekstrak metanol akar kokosan. Ekstrak akar metanol difraksinasi berurutan dengan n-heksana dan diklorometana (DCM). Semua fraksi termasuk ekstrak metanol diuji fitokimia. Fraksi DCM dikromatografi sehingga diperoleh senyawa **1**. Data spektrum DART-HRMS senyawa **1** menunjukkan puncak ion positif pada m/z 469.1861 $[M+H]^+$ (terhitung 469,1862 yang sesuai dengan rumus molekul $C_{26}H_{29}O_8$). Struktur **1** secara lengkap ditentukan dengan NMR 1D (^{1}H-NMR, ^{13}C-NMR, dan DEPT-135) dan 2D (COSY, HSQC, HMBC, dan NOESY), serta dibandingkan dengan data dari literatur.*

Kata kunci: *dukunolide D, kokosan, Lansium domesticum, limonoid, Meliaceae*

INTRODUCTION

Meliaceae is a plant family of mostly trees with large crowns that grows to a height of 30 m with a diameter of about 40 cm. Limonoids are one of the main constituents in this family and are known as biomarker natural products of Meliaceae (Tambunan *et al*. 2021). Zhang & Xu (2017) reported that more than 365 limonoids have been identified from Meliaceae together with their various biological activities. One of the Meliaceae genera which is attractive to be discovered further for limonoid structures is *Lansium*.

The limonoid structures are constructed through oxidative alteration of triterpenoids due to oxidation of the side chain into a substituted furan ring by losing four terminal carbon atoms. Therefore, limonoids are also known as tetranortriterpenoid. Phytochemical investigations into this plant have exhibited terpenoids, alkaloids, flavonoids, and phenolics with diverse biological activities such as anticancer, antibacterial, antimalarial, antifungal, and antiviral (Zulfikar *et al*. 2020; Mayanti *et al*. 2018). Additionally, limonoids can also be used as insecticides (Mayanti *et al*. 2011; Mirnawaty *et al*. 2012) and these compounds showed breast anticancer activity (Purwani *et al*. 2021).

Lansium domesticum is a species which has three varieties known as duku, langsat, and kokosan. These varieties are widely recognized in the local fruit market and can be distinguished mainly on the basis of their fruit morphology (Yulita 2011). However, based on the infraspecies category, the grouping of L. domesticum was divided into duku and kokosan-langsat (Hanum *et al*. 2012). Kokosan has dark green leaves with densely hairy both top and bottom surfaces and the fruits are generally sour.

According to the literature, exploration of secondary metabolites from kokosan has been reported. Tjokronegero *et al*. (2009) obtained two secogammacera isomers from the seeds of kokosan.

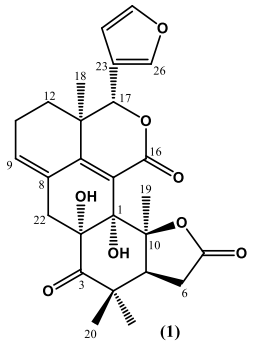

Figure 1. Dukunolide D (**1**)

Mayanti *et al*. (2011) isolated limonoid kokosanolides A and C from the seeds and three onoceranoid-type triterpenoids (kokosanolide B, 8,14-secogammacera-7,14-diene-3,21-dione, and α,γ-onocerandiendione) from the stem bark of kokosan. Furthermore, Mayanti *et al.* (2015) characterized cycloartane-type triterpenoids 9,19-cyclolanost-24-en-3-one and 21,23-epoxy-21,22-dihydroxy (21R, 22S, 23S) from the ethyl acetate fraction of the leaves of kokosan. Kokosanolide D was isolated from fruit peels of kokosan (Fauzi *et al*. 2021). Mayanti *et al*. (2023) obtained kokosanolide G from the seeds and kokosanolides E and F from the fruit peels. Unfortunately, there is no limonoid structures have been reported from the root of kokosan until now. As part of our continuing to study limonoid structures from the Lansium plants, we report for the first time herein the structure of dukunolide D (Figure 1) from methanol extract of the root of kokosan. This study has proved that duku and kokosan are closely related.

MATERIALS AND METHODS

Materials

The root of kokosan was powdered (6.5 kg) using a wood powder machine. Redistilled solvents such as dichloromethane (CH_2Cl_2), ethyl acetate (C_4H_8O2), chloroform ($CHCl_3$), methanol (CH_3OH), and n-hexane (C_6H_{14}) were used for extraction and chromatography. Reagents iron (III) chloride ($FeCl_3$ 5%), Dragendorf, Mayer, Wagner, Liebermann-Burchard, and Shinoda were used for phytochemical testing. TLC analysis was performed on aluminium precoated silica gel plates (Kieselgel 60 F254, 20× 20 cm, 0.25 mm thick, Merck) with detection under UV at 254 and 365 nm. Vacuum Liquid Chromatography (VLC) was carried out on silica gel (Kieselgel 60 G), and open column chromatography (CC) was run on silica gel 60 G (0.04–0.063 mm) as the stationary phase. Spectrometers NMR (Bruker Avance III) HD600 (frequency: 600.13 MHz for ^{1}H-NMR, 150.92 MHz for ^{13}C-NMR and DEPT-135) with Prodigy liquid nitrogen cryoprobe and positive mode DART-HRMS (Thermo Scientific) with an exactive plus orbitrap were employed for structure determination.

Sample Preparation

The root of kokosan was collected from (0º30′01.3″N 110º45′56.5″E), Parus village, Sanggau district, west Kalimantan, Indonesia in July 2022. Species determination was identified (no: 094/A/LB/FMIPA/UNTAN/2022) by a Staff in the Biology Department, Faculty of Mathematics and Natural Sciences, University of Tanjungpura.

Extraction and Isolation

The powder of kokosan root was macerated in methanol (3 x 24 hours). The solvent of MeOH extract was removed by reducing pressure in the rotary evaporator to give a brown residue (141.6 g; 2.2%). A part of the crude extract (67.6 g) was partitioned between n-hexane (2.2 g) and dichloromethane (30.3 g) in order to obtain 3 fractions including the soluble methanol fraction (35.1 g). Each fraction and crude extract were subjected to phytochemical testing. The dichloromethane fraction was fractionated by VLC in increasing polarity of n-hexane: ethyl acetate (7:3, 6:4, 5:5, 4:6, 3:7, 2:8). Six fractions (AA1-AA6) were subsequently obtained on combining the eluates on the basis of TLC. Fraction AA1 (1.9 g) was further purified by normal phase (NP)-column chromatography and subsequently preparative-TLC afforded compound 1 (5 mg). The purity of compound 1 was examined by TLC aluminium plates with two eluents, n-hexane: ethyl acetate (9:1) and chloroform:n-hexane (5:5).

RESULTS AND DISCUSSION

Phytochemical investigations showed that the root of kokosan contained alkaloids, flavonoids, phenolics, as well as terpenoids. Results from phytochemical assays are shown in Table 1.

Structure Elucidation

Compound **1** was obtained as a white amorphous solid soluble in chloroform. DART-HRMS data showed a positive ion peak at *m/z* 469.1861 $[M+H]^+$ (calculated 469.1862) which corresponds to the molecular formula $C_{26}H_{28}O_8$ with thirteen degrees of unsaturation. The ^{1}H-NMR spectrum (Figure 2) and data (Table 2) showed the presence of four tertiary

Table 1. Results of phytochemical testing on the crude extract and fractions of the root of kokosan

Secondary metabolites	Fractions			Crude extract
	MeOH	DCM	*n*-Hex	
alkaloids	-	+	+	+
flavonoids	+	+	+	+
phenolics	+	+	-	+
terpenoids	+	+	+	+

(+) = identified, (-) = did not indentify

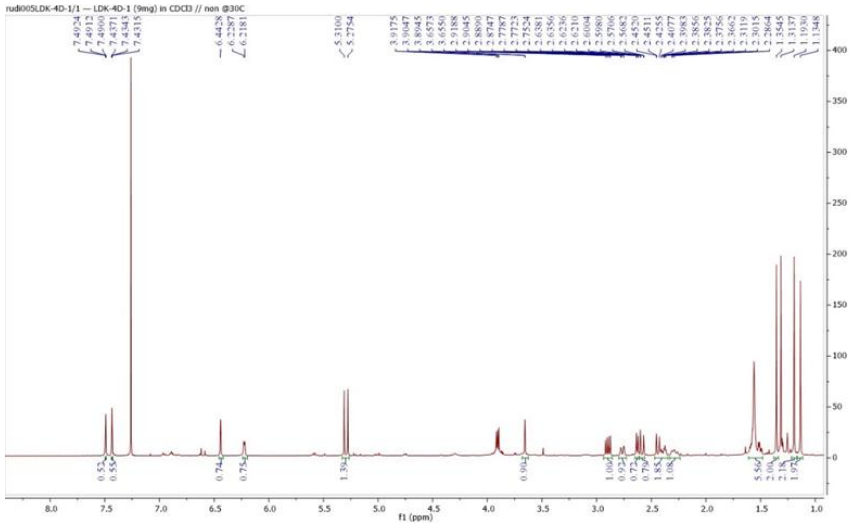

Figure 2. ^{1}H-NMR spectra for dukunolide D **(1)**

methyl groups at δ_H 1.35 ppm, 1.31 ppm, 1.19, and 1.13 ppm (CH_3-19, CH_3-20, CH_3-21, CH_3-18, respectively), four methylene protons at δ_H 2.89 ppm and 2.58 ppm (each for 1H; H-6β/H-6α), 2.76 ppm and 2.41 ppm (each for 1H; H-22β/H-22α), 2.40 ppm and 2.29 ppm (each for 1H, H-11β/H-11α), and 1.52 ppm (2H; H-12), a methine proton at δ_H 2.63 ppm (1H, H-5), one oxygenated methine proton at δ_H 5.29 ppm (1H, H-17), an alkene proton at δ_H 6.22 ppm (1H, H-9), and a broad singlet signal at δ_H 3.65 ppm that indicated the presence of a hydroxyl group. There were three down-field methine protons at δ_H 7.49 ppm (1H, H-26), 7.43 ppm (1H, H-25), and 6.44 ppm (1H, H-24) which are characteristic of a β-substituted furan ring (Saewan *et al*. 2006; Nishizawa *et al*. 1988).

^{13}C-NMR spectrum (Figure 3) and data (Table 2) supported by DEPT-135 revealed twenty-six carbon signals, including four methyl groups at δ_C 22.7 ppm (CH_3-20), 27.5 ppm (CH_3-19), 27.1 ppm (CH_3-21), and 16.5 ppm (CH_3-18), four methylene carbons at δ_C 35.9 ppm (CH_2-22), 31.6 ppm (CH_2-6), 30.1 ppm (CH_2-12), and 22.0 ppm (CH_2-11), and six methine signals at δ_C 143.1 ppm (CH-25), 141.3 ppm (CH-26), 138.7 ppm (CH-9), 109.9 ppm (CH-24), 80.2 ppm (CH-17), and 48.7 ppm (C-5). The ^{13}C NMR spectrum also showed three carbonyl signals at δ_C 214.3 ppm (C-3), 174.2 ppm (C-7), and 164.8 ppm (C-16).

The β-substituted furan ring was constructed based on HMBC correlations from a methine proton at δ_H 5.29 (H-17) to methine carbons at δ_C 109.9 (C-24) and 141.3 (C-26) and to quaternary carbons at δ_C 119.6 and 153.3 assigned for C-23 and C-14, respectively, and from a methine proton at δ_H 7.43 (H-25) to an olefinic carbon at δ_C 141.3 (C-26) and a quaternary carbon at δ_C 119.6 (C-23). The attachment of the β-substituted furan ring at δ_C 80.2 (CH-17) was secured by HMBC correlations (Figure 4) from an oxygenated methine proton at δ_H 5.29 (H-17) to a quaternary carbon at δ_C 119.6 (C-23), an ester carbonyl at δ_C 164.8 (C-16), two olefinic carbons at δ_C 109.9 (CH-24) and 141.3 (CH-26), and from a methyl proton at δ_H 1.13 (H-18) and a methylene proton at δ_H 1.52 (H-12) to an oxymethine carbon at the δ_C 80.2 (CH-17). Two protons at δ_H 2.40 ppm and 2.29 ppm (H-11β/H-11α), methylene protons at δ_H 1.52 ppm (H-12), and an alkene proton at δ_H 6.22 ppm (H-9) were link by COSY data. In addition, two methine protons at δ_H 6.44 ppm (H-24) and δ_H 2.63 ppm (H-5) were COSY correlated to H-25 (δ_H 7.43 ppm) and H-6 (δ_H 2.89 ppm), respectively. The methyl group at δ_C 37.1 (C-13) and a furan ring at δ_C 80.2 (C-17) are *cis* position, which was confirmed by NOESY correlation between the methyl protons at δ_H 1.13 ppm and a methine proton at δ_H 6.44 ppm and X-ray analysis (Fun *et al*. 2006), and it has *R* configuration (Nishizawa *et al*., 1988). The NOESY

Table 2. NMR data of compound **1** in $CDCl_3$ (600 MHz)

No	Compound **1**		COSY(^{1}H-^{1}H)	HMBC ($H \rightarrow C$)
	δ_H (mult, *J* Hz)	δ_C		
1	-	77.0	-	-
2	-	86.7	-	-
3	-	214.3	-	-
4	-	44.1	-	-
5	2.63 (1H dd, 1.5;8.7)	48.7	-	C4, C6, C7, C20, C21
6a	2.58 (1H dd, 8.7;17.9)	31.6	H-6β	C4, C5, C7
6b	2.89 (1H dd, 1.5;17.9)		H-6α,	
7	-	174.2	-	-
8	-	125.9	-	-
9	6.22 (1H, d, 6.2)	138.7	H-11	C11, C12, C22, C14
10	-	76.7	-	-
11a	2.29 (1H, m)	22.0	H-12, H-9	C8, C9, C13
11b	2.40 (1H, m)			
12	1.52 (2H, m)	30.1	H-11a, 11b	C11, C13, C17, C18
13	-	37.1	-	-
14	-	153.3	-	-
15	-	119.9	-	-
16	-	164.8	-	-
17	5.29 (1H, s)	80.2	-	C12, C13, C14, C18, C23, C24, C26
18	1.13 (3H, s)	16.5	-	C12, C13, C14, C17
19	1.35 (3H, s)	27.5	-	C1, C5, C10
20	1.31 (3H, s)	22.7	-	C3, C4, C5, C21
21	1.19 (3H, s)	27.1	-	C3, C4, C5, C20
22a	2.41 (1H, d, 15.8)	35.9	H-22β	C1, C3, C8, C9, C14
22b	2.76 (1H, d, 15.8)		H-22α	
23	-	119.6	-	-
24	6.44 (1H, d, 1.7)	109.9	-	C23, C26
25	7.43 (1H, t, 1.7)	143.1	-	C23, C24
26	7.49 (1H, brs)	141.3	-	C23, C24, C25
OH-2	3.65 (1H, brs)	-	-	C1, C3, C22

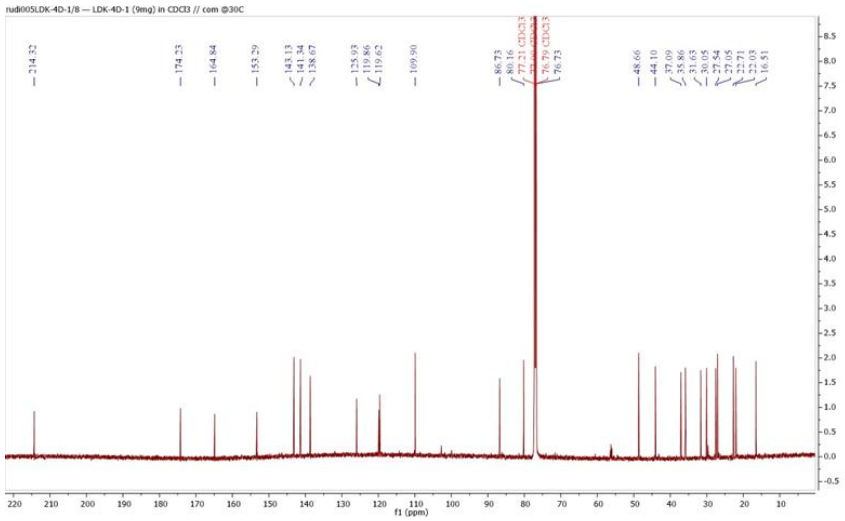

Figure 3. ^{13}C-NMR spectra for dukunolide D (**1**)

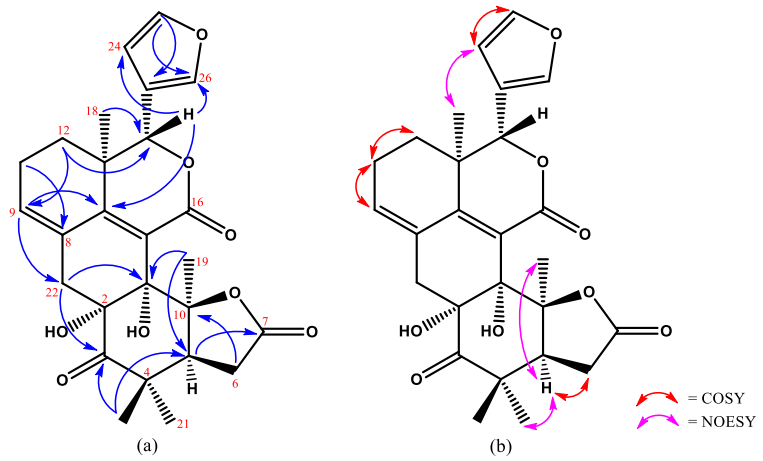

Figure 4. HMBC correlations (a), COSY and NOESY correlations (b) in **1**

spectrum also showed correlations between a methine proton at δ_H 2.63 ppm and two methyl groups at δ_H 1.35 ppm (H-19) and δ_H 1.19 ppm (H-21).

A ketone moiety at C-1 was established by correlations between a geminal proton at δ_H 2.41 (1H, d, *J* = 15.8 Hz, H-22α) and a tertiary methyl signal at δ_H 1.31 (3H, s, H-20) to δ_C 214.3. Further, a γ-butyrolactone ring was constructed by long-range correlations between a secondary methyl proton at δ_H 1.35 (3H, s, H-19) to two oxygenated carbons at δ_C 77.0 and 76.7, assigned to C-1 and C-10, respectively and a methine carbon signal at δ_C 48.7, assigned to C-5. An ester carbonyl at C-7 was established by HMBC correlations between a geminal protons at δ_H 2.89 (1H, dd, *J* = 1.5, 17.9 Hz, H-6β) and 2.58 (1H, dd, *J* = 8.7, 17.9 Hz, H-6α) and a methine proton signal at δ_H 2.63 (1H, dd, *J* = 1.5, 8.7 Hz, H-5) to δ 174.2, assigned to C-7. Comparison of ^{1}H and ^{13}C NMR data between compound **1** and dukunolide D from the literature (Nishizawa *et al*. 1988; Rudiyansyah *et al*. 2018) showed that it was extremely similar. Even though kokosan and duku are different in terms of variety but these plants belong to the same species. Hence, compound **1** was deduced as a known limonoid, dukunolide D. Previously, compound **1** has been tested for antibacterial (*Staphylococcus aureus*, *Enterococcus faecalis*, *E. faecium*, *Acinetobacter baumannii*), anti-TB, and cytotoxic activities (A2780 sens and A2780 CisR), unfortunately, they all exhibited weak activities (Rudiyansyah *et al*. 2018).

CONCLUSION

Dukunolide D (**1**) has been successfully isolated from the root of *L. domesticum* cv kokosan. The structure of **1** was determined by 1D and 2D NMR, DART-HRMS, and compared to NMR data from literature. The β-substituted furan ring is typical for limonoids from *Lansium* plants.

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