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Isolation and Structure Determination of Stigmaterol from the Stem Bark of *Lansium domesticum* Corr. Cv. Kokossan

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Abstract: Indonesia is a tropical country with biodiversity and a source of natural compounds such as terpenoids, phenolic, flavonoids, and alkaloid groups. The steroid is one of the large groups of terpenoid compounds with various structures and bioactivities. The steroid was also commonly found in the Meliaceae family. One species of this family is *Lansium domesticum* Corr. which has been studied to have various activities from isolated compounds (limonoids, sesquiterpenoids, onoceranoids, cycloartanoids, and steroids) as well as from their extracts such as antidiarrheal, antimalarial, antimicrobial, antiinsecticide, antifeedant and were used as traditional medicine such as eye drops and scorpion stings. The *n*-hexane fraction from the bark of *L. domesticum* Cor. cv. Kokosan produced steroid compounds whose structure is determined by IR, ^{1}H-NMR, ^{13}C-NMR, and DEPT spectroscopy methods and compared with the literature. It was concluded that the steroid isolated was (22*E*)-stigmasta-5,22-dien-3ol and was first isolated from this genus. This compound was inactive against MCF-7 breast cancer cells with an IC_{50} value of 832.14 µM.

Keywords: Isolation, Lansium domesticum Corr., Meliaceae, steroid

Abstrak: *Indonesia merupakan negara tropis dengan keanekaragaman hayati dan merupakan sumber penghasil senyawa bahan alam seperti kelompok terpenoid, fenolik, flavonoid dan alkaloid. Steroid merupakan salah satu senyawa golongan besar dari kelompok terpenoid yang memiliki keberagaman struktur dan bioaktivitas. Steroid juga banyak ditemukan di tumbuhan family Meliaceae dan salah satu species dari famili ini yaitu Lansium domesticum Corr. yang telah diteliti memiliki beragam aktivitas dari senyawa yang diisolasi (limonoid, sesquiterpenoid, onoceranoid, sikloartanoid dan steroid) maupun dari ekstraknya seperti antidiare, antimalaria, antimikroba, antiinsektisida, antifeedant dan digunakan sebagai pengobatan tradisional seperti obat tetes mata dan sengatan kalajengking. Bagian n-heksan dari kulit batang L. domesticum Cor. Cv. Kokosan menghasilkan senyawa steroid yang struktur senyawanya ditentukan dengan metode spektroskopi MS, IR, ^{1}H-NMR, ^{13}C-NMR dan DEPT serta dibandingkan dengan literatur maka disimpulkan steroid yang diisolasi yaitu (22E)-Stigmasta-5,22-dien-3ol dan pertama kali diisolasi dari genus ini. Senyawa ini tidak aktif terhadap sel kanker payudara MCF-7 dengan nilai IC_{50} 832.14 µM.*

Kata kunci: *Isolasi, Lansium domesticum Corr., Meliaceae, steroid*

INTRODUCTION

Lansium domesticum Cor. are species and annual plants included in the genus Lansium. *L. domesticum* Corr. grows in the tropics, especially in Southeast Asia, like the Philippines, Malaysia, Thailand, and Indonesia. Previous research on this species reported that this species has compounds that have been isolated as follows: triterpenoids, onoceranoid type, glycosides type, cycloartanoid type, steroid, limonoids, and sesquiterpenoids (Saewan *et al*. 2006; Mayanti *et al*. 2011; Mayanti *et al*. 2015; Marfori *et al*. 2015; Rudiyansyah *et al*. 2018; Fadhilah *et al*. 2020). This species has three cultivars in Indonesia, namely kokosan, pisitan, and duku (Mayanti *et al*. 2015). This plant is often used in traditional medicine, such as eye drops and treatment against scorpion stings. The fruit peels also contained oleoresin, used to treat diarrhea and intestinal spasms. The dried fruit peels in the Philippines are usually burned as a mosquito repellent (Chantrapromma *et*

al. 2004; Lim 2012). Eighty secondary metabolites from this species had been isolated and tested as antimalarial, anti-insecticide, antiplasmodial, antifeedant, and anticancer until 2021 (Tanaka *et al*. 2002; Saewan *et al*. 2006; Marfori *et al*. 2015; Matsumoto *et al*. 2019; Matsumoto & Watanabe 2020).

In addition to diverse bioactivity, one group that has been isolated from this plant is the pregnane and cardenolide steroid types. A pair of geometric isomers of pregnane steroids, namely 17(20)*E*-dyscusin B and 17(20)*Z*-dyscusin B (Ji *et al*. 2021) and isolated from the ethanol extract of *L. domesticum* leaves. While cardenolide steroids were isolated by Tsuchiya *et al*. (2020) and obtained from the ethanol extract of *L. domesticum* leaves. These include obebioside A, obebioside B, honghelin, obeside B, obeside C, and digitoxigenin. The large variety of groups that may be substituted in the main steroid frame or undergo further oxidation caused the steroid group to have a lot of variations. This species has three cultivars, one of which is Kokosan.

MATERIALS AND METHODS

Preparation and Maceration

The wet bark of *L. domesticum* Corr. CV. Kokosan was obtained from the Pangandaran Botanical Gardens, West Java, and determined at Laboratory Plant Taxonomy, Faculty of Mathematics and Natural Science, Universitas Padjadjaran. The bark was dried in the central laboratory of Universitas Padjadjaran. Organic solvents were used including ethanol, ethyl acetate, *n*-butanol, methanol, *n*-hexane, methylene chloride chloroform and acetone purchased from Kristata Gemilang Company, Bandung. The MCF-7 cells were cell culture collections from the Central Laboratory of Universitas Padjadjaran. Roswell Park Memorial Institute-1640 (RPMI-1640) medium, fetal bovine serum (FBS), 0.25% Trypsin-EDTA solution, Penicillin-Streptomycin (PSM) 5000 U/mL, DMSO (Dimethyl sulfoxide), MTT assay kit (CellTiter 96® AQueous One Solution Cell Proliferation Assay from Promega).

Bark (3.18 kg) of mashed kokosan was then extracted using the maceration method with ethanol (20 L) at room temperature. The ethanol extract of bark kokosan was evaporated using rotary evaporator at 40°C under reduced pressure from time to time to obtain a concentrated ethanolic extract residue (300 g). Then this residue was dissolved in water and partitioned with *n*-hexane (8 L) to yield 124 g of *n*-hexane extract, then the water residue was again partitioned with ethyl acetate (7 L) to produce 54 g of ethyl acetate extract, and the same method was carried out for *n*-buthanol (1.5 L) to yield 15 g of *n*-buthanol extract.

Instruments

The IR spectra were recorded on Perkin Elmer Spectrum 100 FT-IR spectrometer. The mass spectra were determined with Waters Q-TOF Xevo mass spectrometer instrument. The NMR spectrum of compound 1 was recorded on JEOL JNM-ECX500R/S1 spectrometer at 500 MHz for ^{1}H and 125 MHz for ^{13}C with TMS as an internal standard. The column chromatography was performed on silica gel 60 (Merck, 70–230 and 230–400 mesh). The TLC analyses were carried out with silica GF_{254} (Merck, 0.25 mm) using various solvent systems. Spot detection was achieved by spraying with 10% H_2SO_4 in Ethanol, followed by heating and heating irradiating under ultraviolet-visible light (254 and 365 nm).

Isolation, Purification, and Elucidation

The *n*-hexane extract (124 g) was fractionated by vacuum liquid chromatography on silica gel G60 using 10% gradient elution of *n*-hexane: ethyl acetate: methanol (from 100% *n*-hexane up to the ratio of the solvent 10:60:30) to acquire 12 (A-L) subfractions. Fraction D (8 g) was further fractionated by vacuum liquid chromatography using 2% gradient elution of *n*-hexane: Ethyl acetate (7:3) to afford four fractions. Fraction D2 (300 mg) was recrystallized with methanol to produce pure isolates (steroids). Pure isolate elucidated using the IR, HR-TOFMS, and NMR spectroscopies.

Cytotoxic Activity Test by the MTT Assay

Cell viability was assessed by MTT reagent (Thermo Fisher Scientific, Uppsala, Sweden) based on reducing resazurin (blue), which works as a function of redox potential. Actively respiring cells convert the water-soluble MTT to an insoluble purple formazan. The formazan is then solubilized, and its concentration is determined by optical density. In the initial step, MCF-7 cell cultures that were 80% confluents were washed with 1 mL 1× PBS twice. Cells were added with 1 mL of trypsin EDTA and then incubated for 3 minutes until the cells were released. Then, the cells were transferred into a Falcon tube containing 5 mL of culture medium and centrifuged at 1200 rpm for 4 minutes. The supernatant was discarded, and cells were resuspended with 1 mL of culture medium. Then, cells were counted using a hemocytometer and plated in 96 well plates with a serial number for the standard curve (6 replications) and 3 repetitions for the treatment Then, 100 µL of the medium was added and incubated for 24 hours at 37°C, 5% CO_2.

The medium was replaced with 180 µL of new medium and added 20 µL of compound 1 (1, 10, 100, 250, and 500 ppm) with co-solvent with various percentages of DMSO (0.5–2.5%) in PBS (Uppsala, Sweden). Incubated for 24 hours, temperature 37°C, 5% CO_2. After that, cells were added with MTT 20 µL, incubated for 3 hours, 37°C, 5% CO_2. The

absorbance was determined at 570 nm, and the IC_{50} value was determined based on the comparison of the percentage of cytotoxicity to untreated cells. In this trial, the used control positive was Doxorubicin. Based on literature (Machana *et al*. 2011; Camarillo *et al*. 2014), all assays and analyses were respectively run in duplicate and all averaged so that a plot of % cytotoxicity versus sample concentration was used to calculate the concentration indicating 50% cytotoxicity (IC_{50}).

RESULTS AND DISCUSSIONS

The *n*-hexane extract of the kokosan stem bark was separated and purified using the column chromatography method to produce Stigmasterol. The steroid compound obtained was in the form of white crystals. The IR spectrum (Figure 1) shows the presence of OH groups (3422 cm^{-1}), aliphatic C-H stretch (2959 cm^{-1}) and C=C (1642 cm^{-1}), which indicates the presence of a C double bond. In addition, there is also 1463 and 1381 cm^{-1} (gem-dimethyl) and 1051 cm^{-1} (C-O stretching). The structural formula was established with HR-TOF-MS (Figure 2), which showed an ion molecular peak of 413.3175 $[M+H]^+$ and calculated for $C_{15}H_{24}O$ m/z 412.2727. This prediction is supported by NMR data. The ^{1}H-NMR spectrum (Figure 3) shows the characteristics of aliphatic compounds where the signal appears in the chemical shift area of 0-2 ppm.

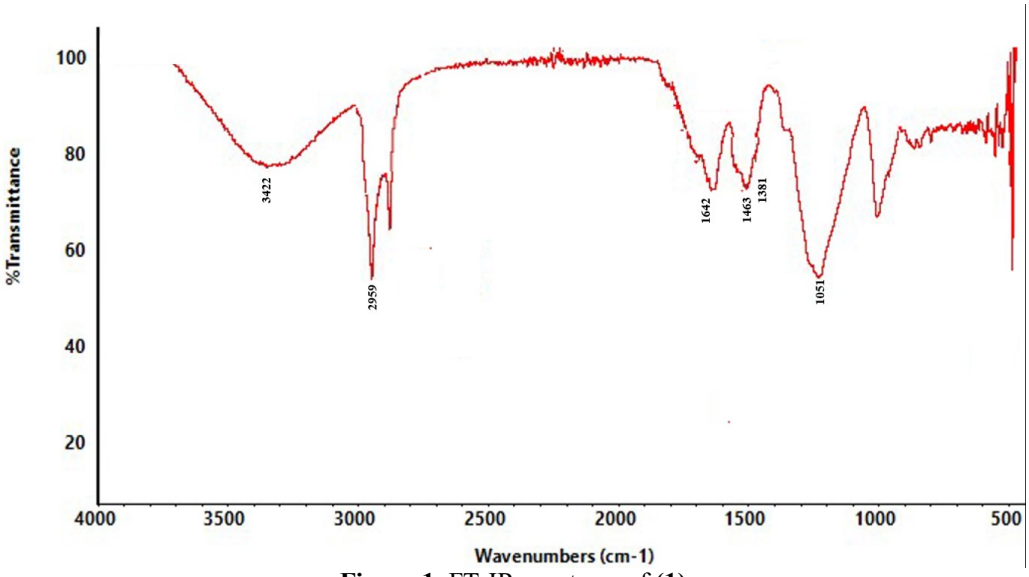

Figure 1. FT-IR spectrum of **(1)**

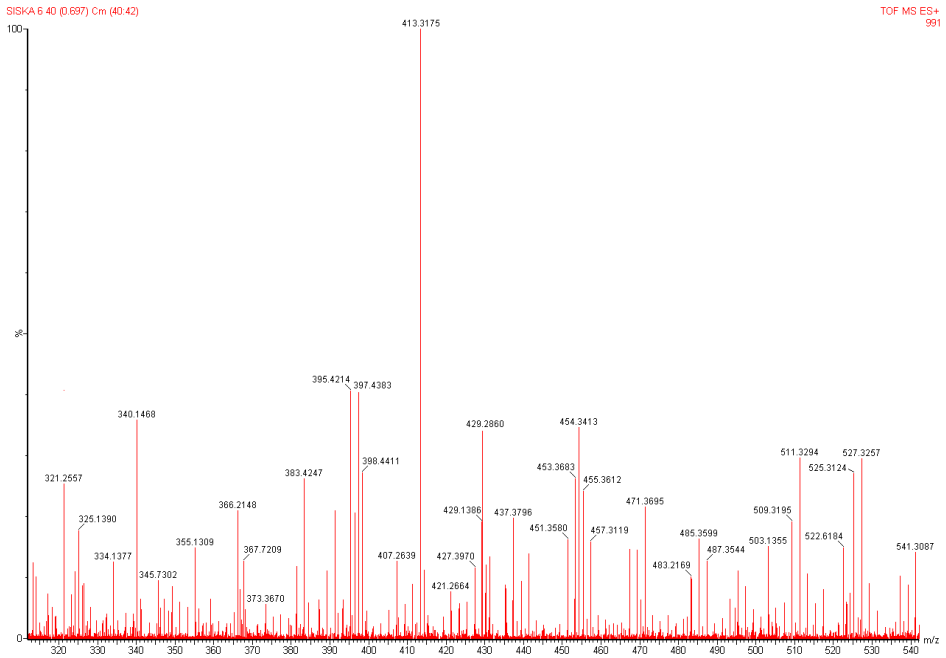

Figure 2. HRTOF-MS spectrum of **(1)**

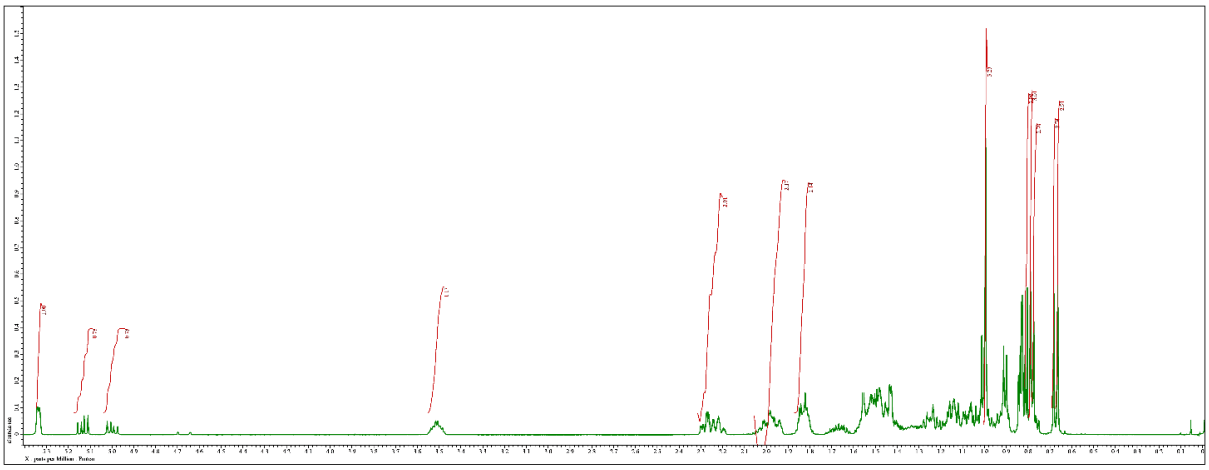

Figure 3. ^{1}H-NMR spectra of **(1)** (500 MHz in $CDCl_3$)

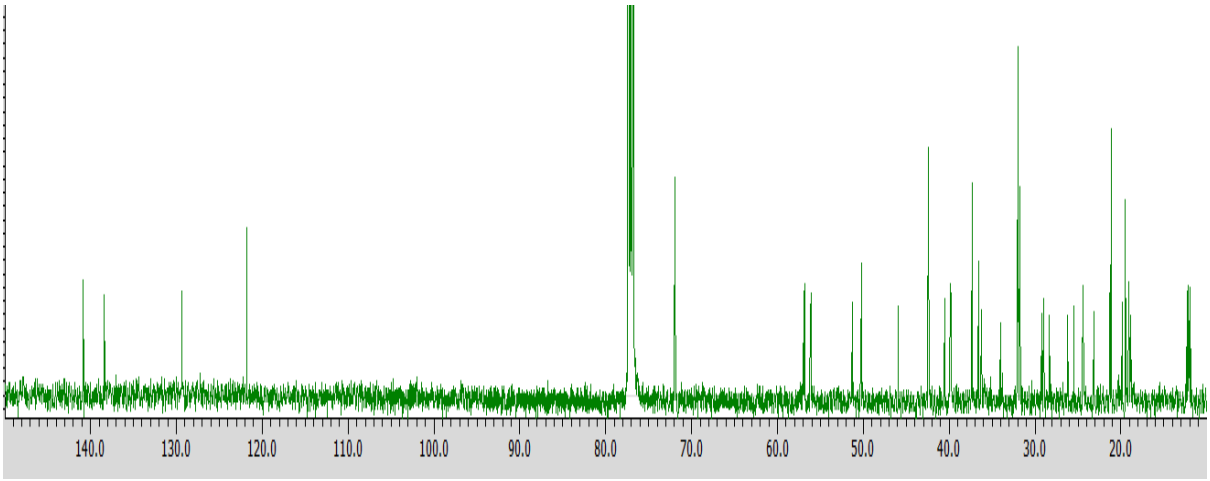

Figure 4. ^{13}C-NMR spectrum of **(1)** (125 MHz in $CDCl_3$)

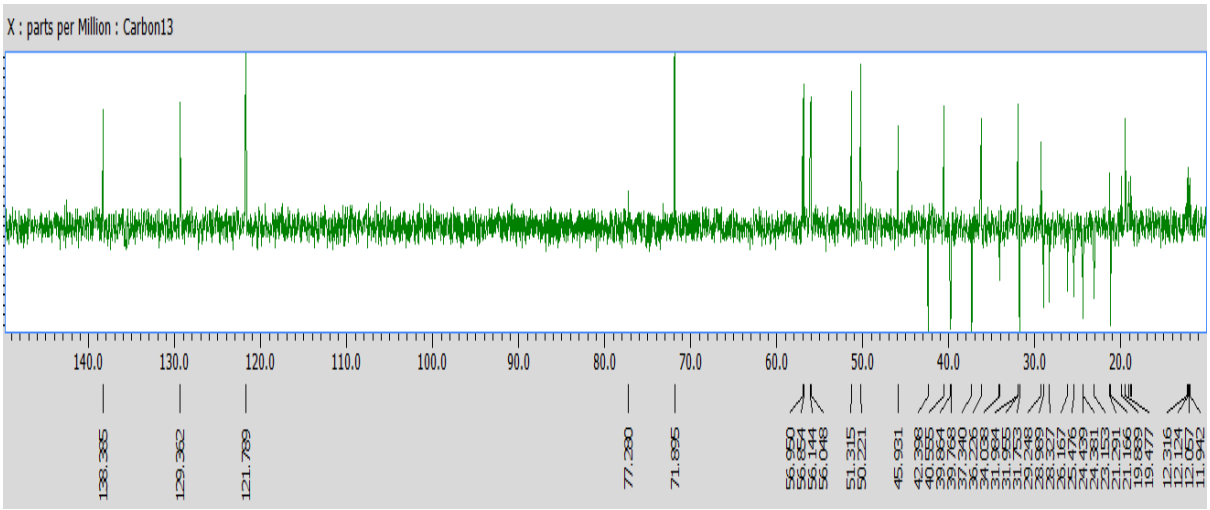

Figure 5. DEPT- 135° spectrum of **(1)** (125 MHz in $CDCl_3$)

The proton signal also indicates the presence of steroidal traits where H-3 appears at a chemical shift of 3.56 ppm with a multiplicity heptet (indicating CH bound to the hydroxyl). Then H-6 appears at a chemical shift of 5.36 ppm, indicating a doublet of doublet (C double-bound CH). This assumption is also supported by the appearance of 2 olefinic protons that have a multiplicity doublet of the

doublet, at shifts of 5.19 and 5.02 ppm. This is identical to the H-22 and H-23 chemical shifts of stigmasterol. In addition, the appearance of two doublet signals at 0.79 (3H, d, 7.2 Hz) and 0.82 (3H, d, 7.2 Hz) can be ascribed to the two methyl groups at H-26 and H-27 and two tertiary methyl protons at 0.65 (H-18) and (H-19) 0.68 ppm.

The ^{13}C-NMR spectrum (Figure 4) shows 29 carbon signals and classification of these signals based on their chemical shift, and the 135° DEPT (Figure 5) ^{13}C-NMR and DEPT spectra showed that the 29 carbons consist of six methyls (two tertiary methyls and gem-dimethyl), nine methylene, 11 methines (one oxygenated carbon and three olefin carbons), and three quaternary carbons (one olefin carbon). Based on the C-NMR of this compound, there are six degrees of unsaturation consisting of two C-C and tetracyclic double bonds. These data have been analyzed, showing the presence of signals at shifts of 140.85 and 121.78 ppm, which are recognized as signals of the C-5 and C-6 double bonds, respectively. The signal that appears at the chemical shift of 71.86 ppm indicates oxygenated carbon, namely carbon bound to hydroxy C-3, which is more deshielded (Achika *et al*. 2016). Another signal appears at the chemical shift of 21.29 ppm and 11.94 ppm, a signal from C-19 and C-18. Both are tertiary methyl but have very different chemical shifts because C-18 undergoes gauche interactions, thereby increasing screening from C-18, and the chemical shift is more shielded (Achika *et al*. 2016). This compound is in agreement with the literature data (Table 1), so it can be concluded that it is 3β,22*E*-sigmasta-5,22-dien-3-ol (Kamboj & Saluja 2011; Achika *et al*. 2016) which was first isolated from the genus Lansium. The chemical structure of stigmasterol is shown in Figure 6.

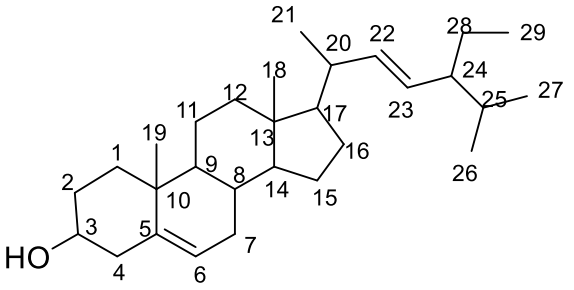

Figure 6. The structure of 3β, 22E-Sigmasta-5, 22-dien-3-ol **(1)**

Plot of % cytotoxicity versus sample concentration was used to calculate the concentration indicating 50% cytotoxicity (IC_{50}). The cytotoxic activity of 3β, 22*E*-Sigmasta-5,22-dien-3-ol was evaluated against the MCF-7 cancer cell lines according to a method described (Xu *et al*. 2015). Cisplatin (53 µM) was used as the positive control. The IC_{50} value of this compound is 832.14 µM.

Table 1. NMR data compound **(1)** ($CDCl_3$ at 500 MHz for ^{1}H and 125 MHz for ^{13}C) compared with compound from literature ($CDCl_3$, 400 MHz)

Carbon Position	CHn	Compounds			
		(1)		Literature (Achika *et al*. 2016)	
		δ_C	δ_H	δ_C	δ_H
1	CH_2	37.26	1.90	37.30	1.90
2	CH_2	31.75	1.53	31.60	1.56
3	CH-OH	71.86	3.54	71.80	3.53
4	CH_2	42.40	2.30	42.37	2.30
5	Cq	140.85	-	140.83	-
6	CH	121.78	5.39	121.7	5.40
7	CH	31.95	1.51	31.90	1.50
8	CH	31.98	2.11	31.95	2.30
9	CH	51.31	0.98	51.25	0.98
10	Cq	36.58	-	36.54	-
11	CH_2	21.16	1.49	21.21	1.50
12	CH_2	39.70	2.07	39.68	2.06
13	Cq	42.29	-	42.30	-
14	CH	56.95	1.06	56.90	1.04
15	CH_2	24.43	1.28	24.40	1.28
16	CH_2	28.32	1.30	28.40	1.30
17	CH	56.14	1.15	56.10	1.16
18	CH_3	11.94	1.10	11.00	1.00
19	CH_3	21,29	0.70	21,2	0.67
20	CH	40.55	1.65	40.50	1.40
21	CH_3	21.20	0.94	21.20	0.91
22	CH	138.39	5.19	138.30	5.16
23	CH	129.30	5.02	129.30	5.05
24	CH	51.20	0.95	51.20	0.97
25	CH	31.90	1.73	31.90	1.71
26	CH_3	21.20	0.86	21.20	0.82
27	CH_3	19.11	0.82	19.00	0.80
28	CH_2	25.47	1.32	25.40	1.31
29	CH_3	12.12	0.91	12.10	0.89

CONCLUSIONS

Steroid 3β, 22*E*-Sigmasta-5, 22-dien-3-ol **(1)**, was isolated from *n*-hexane extracts of the stem bark of L. domesticum Corr. Cv. Kokosan. Steroid **(1)** was reported from the genus Lansium for the first time. This steroid was evaluated for its cytotoxic activity against MCF-7 Breast cancer cell lines showed inactive with IC_{50} value 832,14 µM.

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REFERENCES

Achika, J., Ndukwe, G. & Ayo, R. (2016). Isolation, characterization and antimicrobial activity of 3β, 22*E*-stigmasta-5, 22-dien-3-ol from the aerial part of *Aeschynomene uniflora* E. Mey. *British Journal of Pharmaceutical Research*. **11(5)**: 23506.

Camarillo, I.G., Xiao, F., Madhivanan, S., Salameh, T., Nichols, M., Reece, L.M., Leary, J.F., Otto, K.J., Natarajan, A., Ramesh, A. & Sundararajan, R. (2014). Low and high voltage electrochemotherapy for breast cancer: An in vitro model study. In: Sundararajan, R. (ed). *Electroporation-Based Therapies for Cancer: From Basics to Clinical Applications*. Woodhead Publishing. Oxford. pp. 55-102.

Chantrapromma, K., Saewan, N., Fun, H.K., Chantrapromma, S. & Rahman, A.A. (2004). 6-Hydroxymexicanolide. *Acta Crystallographica Section E: Structure Reports Online*. **60(2)**: o312-o314.

Fadhilah, K., Wahyuono, S. & Astuti, P. (2020). A bioactive compound isolated from Duku (*Lansium domesticum* Corr) fruit peels exhibits cytotoxicity against T47D cell line. *F1000Research*. **9**: 3.

Marfori, E.C., Kajiyama, S.I., Fukusaki, E.I. & Kobayashi, A. (2015). Lansioside D, a new triterpenoid glycoside antibiotic from the fruit peel of *Lansium domesticum* Correa. *Journal of Pharmacognosy and Phytochemistry*. **3(5)**: 140-143.

Ji, K.L., Dai, M.Y., Xiao, C.F. & Xu, Y.K. (2021). Two new steroids with NO inhibitory effects from *Lansium domesticum*. *Natural Product Research*. **35(7)**: 1147-1152.

Kamboj, A. & Saluja, A.K. (2011). Isolation of stigmasterol and β-sitosterol from petroleum ether extract of aerial parts of *Ageratum conyzoides* (Asteraceae). *International Journal of Pharmacy and Pharmaceutical Sciences*. **3(1)**: 94–96.

Lim, T.K. (2012). Lansium domesticum 'Langsat-Lonkong Group'. In: *Edible Medicinal And Non Medicinal Plants*. Springer, Dordrecht.

Machana, S., Weerapreeyakul, N., Barusrux, S., Nonpunya, A., Sripanidkulchai, B. & Thitimetharoch, T. (2011). Cytotoxic and apoptotic effects of six herbal plants against the human hepatocarcinoma (HepG2) cell line. *Chinese Medicine*. **6(1)**: 1-8.

Matsumoto, T., Kitagawa, T., Ohta, T., Yoshida, T., Imahori, D., Teo, S., Ahmad, H.S.B. & Watanabe, T. (2019). Structures of triterpenoids from the leaves of *Lansium domesticum*. *Journal of Natural Medicines*. **73(4)**: 727-734.

Matsumoto, T. & Watanabe, T. (2020). Isolation and structure elucidation of constituents of *Citrus limon*, *Isodon japonicus*, and *Lansium domesticum* as the cancer prevention agents. *Genes and Environment*. **42(1)**: 1-9.

Mayanti, T., Tjokronegoro, R., Supratman, U., Mukhtar, M.R., Awang, K. & Hadi, A.H. (2011). Antifeedant triterpenoids from the seeds and bark of *Lansium domesticum* cv Kokossan (Meliaceae). *Molecules*. **16(4)**: 2785-2795.

Mayanti, T., Sianturi, J., Harneti, D., Supratman, U., Rosli, M.M. & Fun, H.K. (2015). 9, 19-Cyclolanost-24-en-3-one, 21, 23-epoxy-21, 22-dihydroxy (21R, 22S, 23S) from the Leaves of *Lansium domesticum* Corr cv Kokossan. *Molbank*. **2015(4)**: M880.

Rudiyansyah, Alimuddin, A.H., Muharini, R. & Proksch, P. (2018). New tetranortriterpenoids, langsatides A and B from the seeds of *Lansium domesticum* Corr. (Meliaceae). *Phytochemistry Letters*. **23**: 90-93.

Saewan, N., Sutherland, J.D. & Chantrapromma, K. (2006). Antimalarial tetranortriterpenoids from the seeds of *Lansium domesticum* Corr. *Phytochemistry*. **67(20)**: 2288-2293.

Tanaka, T., Ishibashi, M., Fujimoto, H., Okuyama, E., Koyano, T., Kowithayakorn, T., Hayashi, M. & Komiyama, K. (2002). New onoceranoid triterpene constituents from *Lansium domesticum*. *Journal of Natural Products*. **65(11)**: 1709-1711.

Tsuchiya, A., Makita, Y., Koyano, T., Kowithayakorn, T., Ishibashi, M. & Arai, M.A. (2020). Isolation and evaluation of cardenolides from *Lansium domesticum* as notch inhibitors. *Journal of Natural Medicines*. **74(4)**: 758-766.

Xu, M., McCanna, D.J. & Sivak, J.G. (2015). Use of the viability reagent PrestoBlue in comparison with alamarBlue and MTT to assess the viability of human corneal epithelial cells. *Journal of Pharmacological And Toxicological Methods*. **71**: 1-7.