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ON CHEMISTRY, PHARMACY AND
MEDICAL SCIENCES (ICCPM)**

Theme: Advanced Research Development Base on Local Resources

Bengkulu, 27 – 28 November 2018

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Theme: Advanced Research Development Base on Local Resources

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Deni Agus Triawan, S.Si., M.Sc

Reviewer:

Prof. Dr. Irfan Gustian, M.Si

Prof. Dr. Morina Adfa, M.Si

Dr. Eng. Asdim, M.Si

Dr. Agus Martono HP., DEA

Devi Ratnawati, S.Pd., M.Si

Ghufira, S.Si., M.Si

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FOREWORD

Assalamu'alaikum warahmatullahi wabarakaatuh and greetings.

This proceeding contains selected papers of 1st International Conference on Chemistry, Pharmacy, and Medical Sciences (ICCPM) which held on November 26-27, 2018, Santika Hotel, Bengkulu-Indonesia. The conference which was organized by the Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Bengkulu.

The ICCPM 2018 is attended by more than 100 participants. In terms of origin, the participants of this ICCPM are coming from 6 countries i.e. Indonesia, Japan, US, Malaysia, Thailand, and India. The conference is the first international conference organized by the Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Bengkulu and is expected to be held continuously every three years.

The conference particularly encouraged the interaction of research students and developing academics with the more established academic community in an informal setting to present and to discuss new and current work. Their contributions helped to make the conference as outstanding. The papers contributed the most recent scientific knowledge known in the field of Organic Chemistry, Material Chemistry, Pharmacy, Agricultural Chemistry, and Miscellaneous topic related to chemistry.

Our deep gratitude is strongly forwarded to all individuals who took part in the conference, especially the keynote speakers, invited speakers, all the presenters and participants as well as all students and staffs who have been involved in the preparation and execution of the conference and the publication of the proceedings. Our deep gratitude also forwarded for all reviewers the manuscript for this proceedings.

These Proceedings will furnish the scientists with a good reference book. I trust also that this will be an impetus to stimulate further study and research in all these areas.

Bengkulu, 30 November 2018
General Chair of ICCPM
Prof. Dr. Morina Adfa, M.Si



Committee

1st International Conference on Chemistry, Pharmacy and Medical Sciences (ICCPM, Theme: Advanced Research Development Base on Local Resources

Santika Hotel, 27-28 November 2018

Organized by Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Bengkulu

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4. Assoc. Prof. Dr. Sirikantjana Thongmee (Kasetsart University, THAILAND)
5. Assoc. Prof. Dr. Mohammad Abrar Alam (United State of America, USA)

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1. Assoc. Prof. Dr. Mohamad Rafi (Bogor Agricultural University, INDONESIA)
2. Assoc. Prof. Dr. Noor Haida Mohd Kaus (Universiti Sains Malaysia (USM), MALAYSIA)
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4. Assoc. Prof. Dr. Oman Zuas (Research Center for Metrology - LIPI, INDONESIA)

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Isolation and Structure Elucidation of Steroid from Methanol Extract of Sentang (*Azadirachta excelsa* (Jack.) Jacobs) Stem

Angga SANJAYA¹, Morina ADFA^{1*}, Antoni PARDEDE², Irfan GUSTIAN¹, Masayuki NINOMIYA³, Arif Juliari KUSNANDA¹, and Mamoru KOKETSU³

¹Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Bengkulu, Jalan Raya W. R. Supratman, Kandang Limun, Bengkulu 38371, Indonesia.

²Department of Chemistry Education, Islamic University of Kalimantan, Jl. Adhyaksa No. 2 Kayu Tangi, Banjarmasin 70123, Indonesia.

³Department of Chemistry and Biomolecular Science, Faculty of Engineering, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan.

Abstract. A single compound (white powder) has been isolated from methanol extract of Sentang (*Azadirachta excelsa*) stem by means of column chromatography using chloroform and methanol as eluents with step gradient polarity. ¹H-NMR spectrum showed that proton connected with carbon that has hydroxyl and alkene bond. Furthermore, ¹³C-NMR, DEPT 45 and 135 spectrums showed that the 29 carbon atoms comprises of 6 CH₃, 11 CH₂, 9 CH and 3 quaternary carbon. Also, MS spectra proved that an isolated compound has a relative molecular mass m/z 414. Based on data ¹H-NMR, ¹³C-NMR, DEPT 45 and 135, MS and compared with the literature, the isolated compound suggested as β-sitosterol.

Keywords: *Azadirachta excelsa* (Jack.) Jacobs, Chromatography, NMR, MS and steroid.

A. Introduction

Azadirachta excelsa (Jack.) Jacobs locally known as Sentang is a species from *Meliaceae* family. It is distributed all over Sumatra, Kalimantan, Sulawesi, Malaysia, Filipina, Aru Islands and New Guinea. The tree can grow up to 50 m tall, and bole up to 125 cm in diameter. Leaves have a characteristic, up to 60 (-90) cm long, with 7-11 pairs of leaflets. Leaflets asymmetrical, lanceolate to elliptical, up to 12.5 cm long and 3.5 cm, wide, margin entire. Its flowers are small greenish white in up to 70 cm long panicles [1].

In previous studies, the extract from leaves of *A. excelsa* has been reported to be anti-diabetic which has the capacity of lowering *Glycosylated Haemoglobin* (HbA1c), increasing the percentage of plasma insulin and reduce atrophy in the diabetic white rat [2]. The methanol extract of *A. excelsa* wood have potent antifeedant and toxicity against *Crocidolomia binotalis* [3]. In addition, methanol and *n*-hexane extracts of *A. excelsa* seed kernels is known as anti *Aedes aegypti* activity with LC₅₀ value 403.6 and 518.7 ppm [4]. The major compounds in the essential oil of Sentang Seeds are oleic acid (31.3%), hexadecanoic acid (14.2%), octadecanoic acid (13.0%), 4-octylphenol (9.7%), and *O*-methylximedecanal (6.8%) [5].

Phytochemical screening of leaf extract from Sentang has shown that it contains flavonoids, tannins, triterpenes, steroids, and fatty acid [6]. Steroid compound has been reported to exhibit bioactivities such as anti-bacterial, anthelmintic, antitumor [7-9], and impeding cholesterol transfer across the mitochondrial membrane [10].

Based on literature search, so far, there has been no report on contents of steroid compounds from methanol extracts of Sentang stem. Therefore, it is necessary to conduct a research to isolate the steroid compound from the methanol extract of Sentang stem and determine its molecular structure by spectroscopy.

B. Results and Discussion

White powder (22 mg) were isolated from the methanol extract of *A. excelsa* by several chromatography methods. The compound tested by Thin Layer Chromatography (TLC) with UV 254 nm and Ce(SO₄)₂ 1% in H₂SO₄ 10% and it showed a single spot. Phytochemical analysis of the isolated compound with Lieberman Burchard test confirmed a steroid compound.

* Corresponding author: morinaadfa@yahoo.com

It was observed from the results of ¹³C-NMR spectrum analysis that the isolated compound has 29 varieties of carbons. The ¹³C-NMR spectrum (Fig. 1) expresses the presence of hydroxyl group (δ C-3 = 71.89) and alkene carbons appeared at δ 121.8 (C-6) and δ 140.85 (C-5). ¹³C-NMR (100 MHz, CDCl₃, δ) result showed that 11.94 (C-18), 12.07 (C-29), 18.86 (C-21), 19.11 (C-26), 19.49 (C-19), 19.91 (C-27), 21.17 (C-11), 23.15 (C-28), 24.39 (C-15), 26.15 (C-23), 28.33 (C-16), 29.23 (C-25), 31.74 (C-2), 31.99 (C-7), 32.0 (C8), 34.03 (C-22), 36.23 (C-20), 36.23 (C-10), 37.34 (C-1), 39.86 (C-12), 42.38 (C-4), 42.38 (C-13), 45.92 (C-24), 50.21 (C-9), 56.14 (C-17), 56.85 (C-14), 71.89 (C-3), 121.80 (C-6), 140.85 (C-5). Furthermore, to determine the type of carbon group in the compound that analysed using *Distortionless Enhancement by Polarization Transfer* (DEPT). The data showed the presence of 6 methyls (CH₃), 11

methylenes (CH₂), 9 methines (CH), and 3 quaternary carbons.

The ¹H-NMR (Fig. 2) spectrum of the isolated compound showed the presence of six methyl signals that appeared as two singlets at δ 0.68 (3H, H-18) and δ 1.01 (3H, H-19) assignable to two tertiary methyl group at C-18 and C-19 respectively. Three methyl doublets that appeared at δ 0.81 (3H, H-26, J = 6.84 Hz), δ 0.84 (3H, H-27, J = 7.36 Hz), and δ 0.92 (3H, H-21, J = 6.4 Hz) which attributed to three methyl groups at C-26, C-27, and C-21, respectively. On the other hand, the triplet at δ 0.86 (3H, H-29, J = 6.4 Hz) suspected to be a methyl group on C-29. One olefinic proton at δ 5.35 (1H, H-6, broad doublet, J = 5.04 Hz), and a proton multiplet at δ 3.51- 3.54 (1H, H-3) connected to C-6 and C-3.

Fig. 1. ¹³C-NMR (100 MHz, CDCl₃) spectrum of isolated compound.

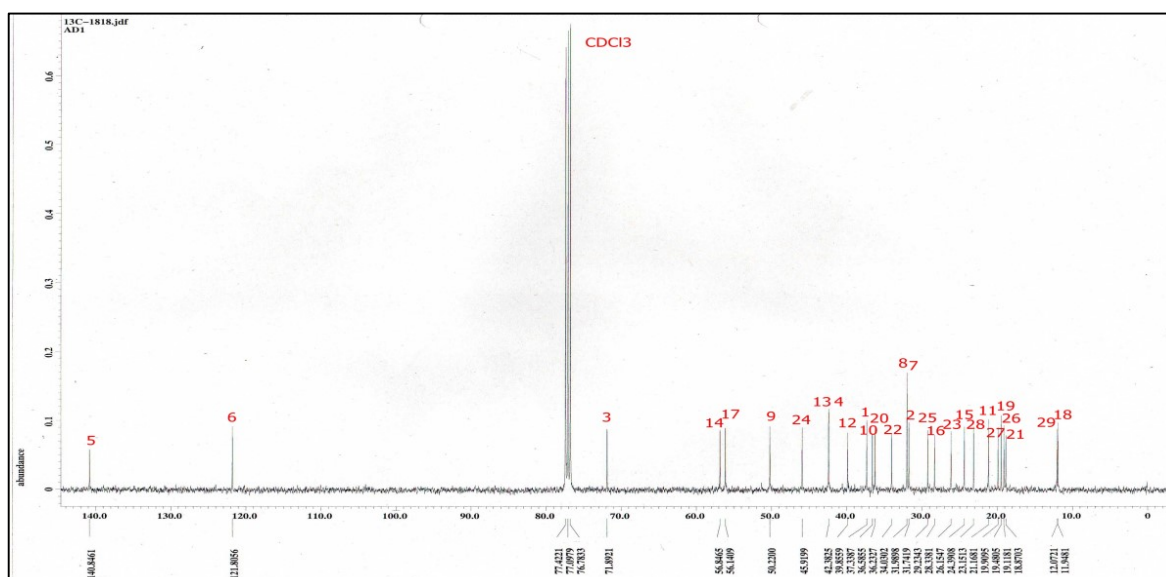


Fig. 2. ¹H-NMR (400 MHz, CDCl₃) spectrum of isolated compound.

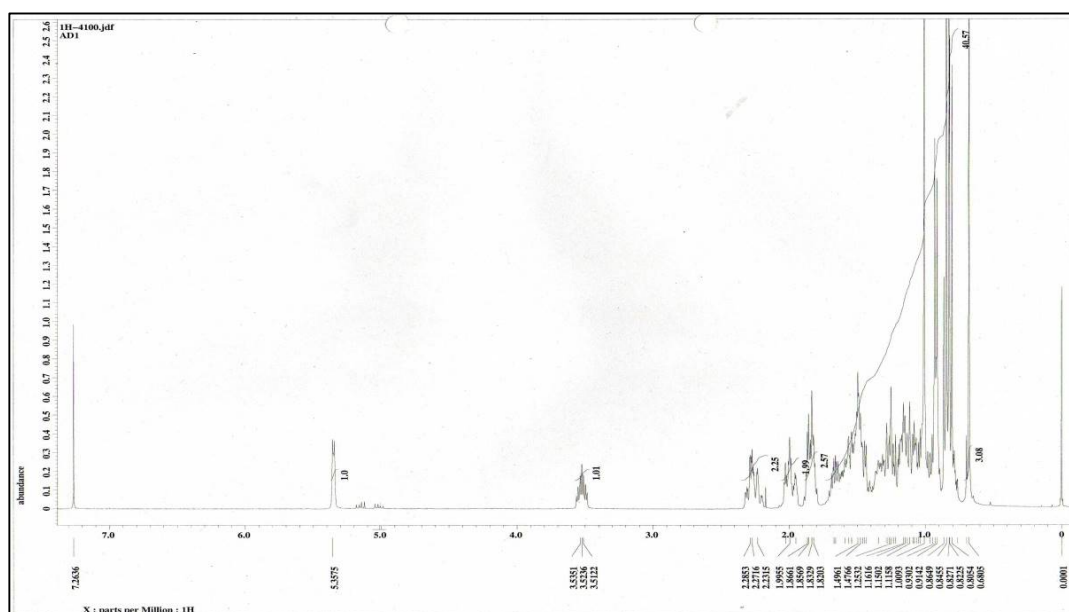
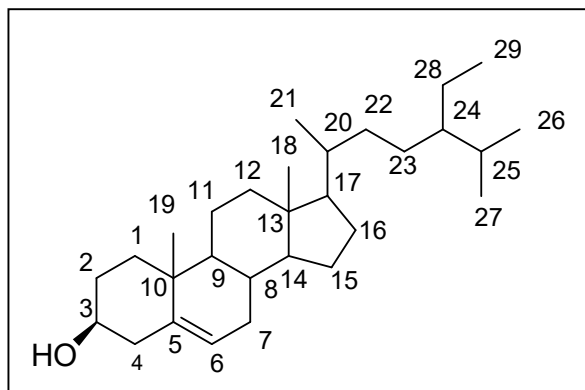


Fig. 3. β -Sitosterol.



The mass spectrum of the isolated compound which gives a peak at m/z 414 is thought to be the peak ion of the molecule $[M^+]$ and molecular formula $C_{29}H_{50}O$. The ionic peak seen at m/z 399 is derived from methyl termination on the side chain, while the ionic peak at m/z 396 comes from the expenditure of water molecules. The relative intensity of several other major fragments is at m/z 396, 303, and 273.

Isolated compound was characterized by 1H -NMR, ^{13}C -NMR, DEPT 45 and 135, MS spectral data and comparison with the literature [11-13], the structure of the isolated compound identified to be β -sitosterol. The isolation of the others compounds from the methanol extract of *A. excelsa* stem and seed are in going.

C. Conclusion

A single compound (white powder) of methanol extract of Sentang (*Azadirachta excelsa* (Jack.) Jacobs) has been isolated using column chromatography. Based on spectrum analysis data of 1H -NMR, ^{13}C -NMR, DEPT 45, 135 MHz, MS and being compared with the literature review, the obtained compound is a steroid group with the name β -sitosterol with has a relative molecular mass of 414 and a molecular formula of $C_{29}H_{50}O$.

D. Experimental Section

4.1. General Experimental

1H and ^{13}C -NMR spectra were recorded on a JOEL ECA 400 spectrometer. MS spectra were recorded using a JOEL JMS-700/GI mass spectrometer. Silica Gel 60 N, neutral 40-50 μm , (KANTO Chemical Co., Inc.) was used for column chromatography. Solvents used were *n*-hexane, chloroform, ethyl acetate, acetone, and methanol from WAKO.

4.2. Plant Material

The fresh stem of Sentang was collected from Bengkulu, Indonesia, while the plant species was identified at Herbarium Bogoriense, Cibinong, Indonesia.

4.3. Procedures

Fresh stem of Sentang (3.5 Kg) were macerated by methanol (16 L) and this process was repeated three times. The methanol extract was evaporated using a rotary evaporator and 55 g of brownish concentrated extract was obtained. Afterwards, a portion of crude methanol extract (45 g) was partitioned by vacuum liquid chromatography with solvent system step gradient polarity using *n*-hexane: $CHCl_3$ (10:1), *n*-hexane: $CHCl_3$ (1:1), $CHCl_3$ (100%), $CHCl_3$: EtOAc (5:1), $CHCl_3$: EtOAc (1:1), EtOAc: acetone (5:1), acetone (100%), and acetone: MeOH (5:1), by 500 mL respectively. This process obtained 7 fractions (M1-M7). M3 fraction (13.03 g) was further subjected to column chromatography (CC) on silica gel eluted with $CHCl_3$ -MeOH (10:0 to 0:10) and obtained nine sub fractions (M3.1-M3.9). CC on silica gel was performed on sub fraction M3.7, which was eluted with $CHCl_3$ -MeOH (10:0 to 0:10), to give 5 sub-fractions (E3.7a-E3.7e). White powder (22 g) was obtained from sub fraction M3.7c. Sub fraction M3.7c showed a single spot in TLC visualization by UV lamp 254 nm and $Ce(SO_4)_2$ 1% in H_2SO_4 10% [14]. The structure of isolated compound was determined based on 1H -NMR, ^{13}C -NMR, DEPT 45 and 135, MS, and compared with literature data.

E. Acknowledgments

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