Jurnal Kimia Valensi, Vol 8(2), November 2022, 263-268
Available online at Website: http://journal.uinjkt.ac.id/index.php/valensi

Nimonol from *Chisocheton macrophyllus* (Meliaceae) Seeds and Their Cytotoxic Activity against P-388 Leukaemia Cells

Nurlelasari[1*], Desi Harneti P. Huspa[1], Rani Maharani[1], Darwati[1], Tri Mayanti[1], Kindi Farabi[1], Muhammad Hanafi[2], Unang Supratman[1]

[1]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Padjadjaran University, Jl. Raya Bandung-Sumedang Km. 21 Jatinangor, 45363, Indonesia
[2]Research Center for Advanced Chemistry, National Research and Innovation Agency (BRIN), Jl. Raya Puspiptek-Serpong, South Tangerang, Banten, Indonesia

**Corresponding author*: *nurlelasari@unpad.ac.id*

Received: August 2022; Revision: September 2022; Accepted: November 2022; Available online: November 2022

Abstract

The *Chisocheton* genus belongs to the Meliaceae family which produces various structures and activities of compounds, such as antimalarial, antimicrobial, antitumor, anti-inflammatory, and cytotoxic. This plant has 53 species that are spread in tropical and sub-tropical forests, including Indonesia. *Chisocheton* plants have been known as plants that produce limonoids, namely triterpenoid compounds that have been modified to lose four terminal carbons (tetranortriterpenoids). One of the species whose phytochemical reports are still few and interesting for research on limonoid content is *Chisocheton macrophyllus*. *Chisocheton macrophyllus* is a tall plant that grows in the rainforest in the northern part of the island of Sulawesi, Indonesia, has the regional name ma aa, gula, pasak lingga, gending, ta suea, bekak, or pithraj tree. This paper will describe a limonoid compound, namely nimonol which has been isolated from *Chisocheton macrophyllus*. Nimonol is known to have the molecular formula $C_{28}H_{36}O_5$ from a group of havanensin. The structure was determined by spectroscopic methods UV, IR, ^{1}D-NMR (^{1}H-NMR, ^{13}C-NMR, and DEPT), 2D-NMR (^{1}H-^{1}H COSY, HMQC, and HMBC), and mass spectroscopy.

Keywords: *C. macrophyllus*, havanensin, limonoid, Meliaceae.

DOI: *10.15408/jkv.v8i2.27782*

1. INTRODUCTION

Chisocheton plants are among the second largest in the Meliaceae family, a family that has been known as a plant that produces various secondary metabolites and interesting biological activities. This plant has 53 species distributed in tropical and subtropical forests such as Asia, Malaysia, and Indonesia (Shilpi *et al*., 2016). *Chisocheton* compounds have been widely reported to have a variety of activities, such as antimalarial (Nurlelasari *et al*., 2016), antimicrobials (Nurlelasari *et al*., 2021; Fitriana *et al*., 2021; Nurlelasari *et al*., 2020; Rahmayanti *et al*., 2021), antifeedant, growth regulator, repellent, larvacide (Nurlelasari *et al*., 2017; Lin *et al*., 2021; Lin *et al*., 2022), antitumor (Rahmayanti et al., 2021; Cahn *et al*., 2012; Najmuldeen *et al*., 2012) and anti-inflammatory (Yang *et al*., 2012) and cytotoxic activity (Nurlelasari *et al*., 2016; Badria & Ahmed., 2018; Katja *et al*., 2016; Nurlelasari *et al*., 2021).

In a previous study of the *Chisocheton* genus, the highest number of limonoids and triterpenoids were found (Shilpi *et al*., 2016; Najmuldeen *et al*., 2012; Supratman *et al*., 2020; Nurlelasari *et al*., 2021; Nurlelasari *et al*., 2015), then steroid, phenolic (Hidayat *et al*., 2018) and spermidine alkaloids (Nguyen *et al*., 2020).

Chisocheton macrophyllus is an Indonesian tropical forest plant whose secondary metabolite studies are still rare, so there is a great opportunity to obtain its limonoid compounds. *C. macrophyllus* is a higher plant found growing in the rain forest in the northern part of Malaysia and Indonesia (Shilpi *et al*., 2016; Nurlelasari *et al*., 2017;

Copyright©2022, Published by Jurnal Kimia Valensi
P-ISSN: 2460-6065, E-ISSN: 2548-3013

Harneti Desi *et al*., 2018). This plant has a local name, a Ma-aa in Indonesia, and the oil from the Chisocheton plant is traditionally used for lighting, while the wood is rarely used (Shilpi *et al.*, 2016).

In the leaves of *C. macrophyllus* (Meliaceae), it was reported that a new triterpenoid was found namely, 24-hydroxydammara-20,25-dien-3-one, and three known triterpenoid compounds, namely moronic acid, oleanolic acid, and betulonic acid which had antitumor activity (Inada *et al*., 1993), in the seeds of *C. macrophyllus* one new limonoid (disobinol) and three known limonoid compounds (disobinin, nimonol, and 7*α*-hydroxyneotricilenon) were isolated (Nurlelasari *et al*., 2017; Rahmayanti *et al*., 2021 and Nurlelasari *et al*., 2021) triterpenoids (Nurlelasari *et al*., 2015), and phenolic compounds have been found (Katja *et al*., 2015). This paper will describe the elucidation of the structure of nimonol, a limonoid that was first discovered in *C. macrophyllus* seeds.

2. MATERIALS AND METHODS

Materials and Tools

Seeds of *C. macrophyllus* were obtained from the Bogor Botanical Gardens, Bogor, Indonesia, in August 2011. This plant was determined by its herbarium without specimen code No. Bo-1295452.

General Experimental Procedures

The equipment used in this study includes glassware commonly used in isolation techniques in the Natural product. In addition, other supporting equipment is used, such as an analytical balance, a Buchi R-200 rotary evaporator with a Buchi V-500 vacuum pump, a Buchi B-490 water bath, and an F-100 cooling circulator. Thin layer chromatography (TLC) and UV Vilber Lourmat detector lamp (λ 254 nm and 365 nm) with 10% (v/v) sulfuric acid in ethanol and Ehrlich's reagent as stain visible reagent. The isolate was analysed and characterised based on absorption spectroscopic infrared (IR) Perkin-Elmer spectrum-100 FT-IR in the KBr plate. Mass spectra were obtained with a JEOL JMS-700 and SynaptG2 mass spectrometer instruments, and NMR spectra were measured with a JEOL JNM ECA-500 spectrometer at 500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR with TMS as standard.

Extraction and Isolation

Seeds powder of *C. macrophyllus* (3.5 kg) was extracted with MeOH at room temperature to obtain a concentrated extract of 414 g of methanol. Then, the concentrated MeOH extract was partitioned successively using *n*-hexane, EtOAc, and *n*-butanol to obtain 197 g, 108 g and 8 g of concentrated extracts of *n*-hexane, EtOAc and *n*-butanol, respectively. Each extract was guided for the presence of limonoids using the Ehrlich stain reagent, producing a red colour indicating the presence of limonoids. The *n*-hexane extract (60 g) was separated by vacuum liquid chromatography (KCV) method and eluted with *n*-hexane, EtOAc and 10% methanol to obtain seven fractions (A01-A7). The A03-A05 fractions were combined (10.5 g) and were separated using column chromatography with the *n*-hexane/EtOAc solvent system (7:3) to obtain 9 subfractions (B01-B9). Subfractions B04 and B05 were combined (0.2 g) and were separated again using column chromatography with a chloroform-acetone solvent system (9.8:0.2) to give nimonol (16.1 mg).

Cytotoxic Assay Against P-388 Murine Leukaemia Cells

The activity test of isolated compounds was determined by testing their cytotoxic properties against murine leukaemia P-388 cells using the Alley method. P-388 murine leukaemia cells are used in the initial screening for compounds that have the potential as anticancer at the National Cancer Institute (NCI). The working principle of the cytotoxic measurement of P-388 murine leukaemia cells is as follows: the activity of pure compounds and artonin E (0.03 g/mL) as a comparison compound is expressed by the determination of IC_{50}, which is the concentration of the sample or comparison required to inhibit 50% of P-388 murine leukaemia cancer cells by staining with MTT reagent [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazoliumbromide; also called bluethiazole], which was observed with a microplate reader at 540 nm.

The test was carried out by adding various concentrations of nimonol and artonin E to murine P-388 cancer cells. After being incubated for 48 hours, MTT colour reagent was added to the sample and incubated for 4 hours. Cells still alive will change the colour of the MTT from yellow to blue. The number of

cancer cells inhibited by the sample can be measured for absorption after adding a stop solution using a microplate reader at 540 nm. The IC_{50} value can be calculated by extrapolating the 50% positive control absorption line on the absorption curve for various sample concentrations on semi-logarithmic paper (Sahidin *et al.*, 2005).

Determination of cytotoxic activity against cancer cells according to Cao *et al*. (1998) with the following categories: very active (IC_{50} < 5 g/mL), active (IC_{50} 5-10 g/mL), moderate (IC_{50} 11-30 g/mL) and inactive (IC_{50} > 30 g/mL).

3. RESULTS AND DISCUSSION

Nimonol

Colourless crystals; UV (MeOH) 240 (ε_{maks} 27.349); IR (KBr) ν_{max} 3414, 1739, 1658, 1450, 1382, 1361 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1; HR-ESI-TOFMS *m/z* 453.2596 $[M+H]^+$, (calcd for $C_{28}H_{36}O_5$, 452.5824).

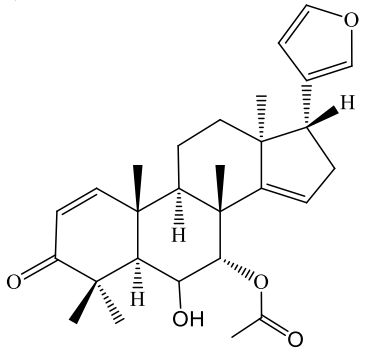

Figure 1. Structures of nimonol

Obtained Nimonol (**Figure 1**) is a colourless crystal soluble in chloroform and acetone. The molecular ion obtained $[M+H]^+$ at *m/z* 453.2596 (calc. for $C_{28}H_{36}O_5$, 452.5824) in the HR-ESI-TOFMS spectrum, showing the molecular formula of $C_{28}H_{36}O_5$, with eleven degrees of unsaturation. UV spectrum analysis showed the presence of the α,β-unsaturated ketone system, is at λ_{max} 240 nm. In contrast, analysis based on the IR spectrum shows the presence of functional groups of hydroxyl (OH) (ν_{max} 3414 cm^{-1}), ester carbonyls (ν_{max} 1739 cm^{-1}), conjugated carbonyl (ν_{max} 1658 cm^{-1}) and olefinic (ν_{max} 1450 cm^{-1}).

The existence of the *α,β*-unsaturated ketone system was confirmed from the ^1H-NMR spectral data, which appeared at the δ_C 205.2 ppm and two olefinic protons [δ_H 5.89 (1H; d; 10.4); 7,10 (1H; d; 10,4)]. Furthermore, the suspicion of limonoid compounds was shown based on the ^1H-NMR spectrum data, namely the presence of five tertiary methyl groups (δ_H 0.83, 1.19, 1.19, 1.19, and 1.30, each 3H, s), as well as the characteristics of the furan group, namely the chemical shift of *β*-furan moiety at [δ_H 6.28 (1H, d, J= 1.3 Hz), 7.26 (1H, s), and 7.39 (1H, m)] (Nurlelasari *et al*., 2017; Rahmayanti *et al*., 2021). There are acetoxyl groups (δ_H 2.18; 3H, s), two oxygenated proton (δ_H 4.07 and 5.46), and one olefinic protons at [5.57 (1H, d, J=1.9 Hz)].

The ^1H-NMR spectrum (**Table 1**) showed the presence of five tertiary methyl groups (δ_H 0.83, 1.19, 1.19, 1.19, and 1.30, each 3H, s), one acetoxyl group (δ_H 2.18; 3H, s), two oxygenated protons (δ_H 4.07 and 5.46), *β*-furan moiety at [δ_H 6.28 (1H, d, *J*= 1.3 Hz), 7.26 (1H, s), and 7.39 (1H, m)], and two olefinic protons at [δ_H 5.89 (1H, d, *J*=10.4 Hz) and 5.57 (1H, d, *J*=1.9 Hz)].

Table 1. NMR data (500 MHz for ^1H and 125 MHz for ^{13}C, in $CDCl_3$) for nimonol

Position H/C	Nimonol	
	δ_H ,(mult, *J* Hz)	**δ_C (mult, *J* Hz)**
1	7.10 (1H, d, 10.4)	157.4 (d)
2	5.89 (1H, d, 10.4)	126.4 (d)
3	-	205.2 (q)
4	-	41.3 (q)
5	2.24 (1H, d,12.0)	48.5 (d)
6	4.07 (1H, m)	68.7 (d)
7	5.46 (1H, d, 2.34)	78.7 (d)
8	-	45.1 (q)
9	2.29 (1H, m)	35.8 (d)
10	-	42.3 (q)
11	1.73 (1H, m)	16.4 (t)
12	1.93 (1H, m)	32.4 (t)
13	-	47.4 (q)
14	-	159.3 (q)
15	5.57 (1H, d, 1.9)	120.8 (d)
16	2.43, 1.67 (1H, m)	34.6 (d)
17	2.86 (1H, dd, 18.5)	51.8 (d)
18	0.83 (3H, s)	20.8 (s)
19	1.30 (3H, s)	27.9 (s)
20	-	124.4 (q)
21	7.26 (1H, s)	139.9 (q)
22	6.28 (1H, d, 1.3)	111.2 (q)
23	7.39 (1H, m)	142.9 (q)
28	1.19 (3H, s)	20.8 (s)
29	1.19 (3H, s)	31.9 (s)
30	1.19 (3H, s)	20.8 (s)
1’	2.18 (3H, s)	21.9 (s)
2’	-	171.7 (q)

Based on the ^{13}C-NMR data detailed by DEPT and HMQC, it can be seen that there are twenty-eight carbon resonances (can be seen in **Table 1**) consisting of one carbonyl (δC 205.2), one acetyl carbon (δ_C 21.9 and 171.7), six sp^2 methines (δ_C 111.2, 124.4, 126.4, 139.9, 142.9, and 157.4), five methyls, two sp^3 methylenes, two sp^3 oxygenated carbons (δ_C 68.7 and 78.7), five sp^3 methines, four sp^3 quarternary carbons, and two sp^2 quarternary carbons (δ_C 124.4 and 159.3). From these data, it can be seen that the functionality is six out of eleven degrees of unsaturation, so there are five degrees of unsaturation remaining which correspond to the presence of pentacyclic limonoids (Nurlelasari *et al*., 2017).

The ^{1}H-NMR and ^{13}C-NMR data for the isolated compound was based on the characteristics of limonoid compounds, which had a furan group and five tertiary methyl groups as the main alkyl groups in limonoids. The difference with the azadiradiron compound is the presence of an OH group at C-6 of this compound. There is also no acetyl signal at C-6 (C 21.9 and 78.7) for the dysobinin compound (Nurlelasari *et al*., 2017), indicating that the isolated compound is nimonol.

To determine the connectivity of the partial structure due to hydroxyl groups, ^{1}H-^{1}H COSY and HMBC experiments were carried out, and the results are shown in **Figure 2**. In the HMBC spectrum, the hydroxyl signal (δ_H 4.07) showed ^2J correlations with C-7 (δ_C 78.7) and methines carbon C-9 (δ_C 35.8). Methyl proton (δ_H 2.18) showed ^2J correlations with C-2' (δ_C 171.7), and methines proton (δ_H 5.46) showed ^2J correlations with C-2' (δ_C 171.7), indicating that the acetyl group was located at C-7. Methine proton (δ_H 7.10) showed ^2J correlations with C-9 (δ_C 35.8), quarternary carbon C-3 (δ_C 205.2), and quarternary carbon C-4 (δ_C 41.3), indicating that olefinic was located at C$\Delta^{1,2}$. Methine proton (δ_H 5.57) showed ^3J correlations with C-17 (δ_C 51.8), and ^2J correlations with C-14 (δ_C 159.3) and methylene carbon C-16 (δ_C 34.6) indicated that olefinic was located at C$\Delta^{14,15}$.

Therefore, the compound was established as a limonoid named nimonol. The stereochemistry of nimonol was determined by the biogenesis of the compound containing the oxygenated proton position at C-7 (Irungu, 2014), and all conformed to the proposed structure. Thus, the structure of the limonoid compound was identified as nimonol (Nurlelasari *et al*., 2017; Laphookhieo *et al*., 2008) based on MS and NMR spectral data and compared the spectrum data with compounds that had been isolated previously.

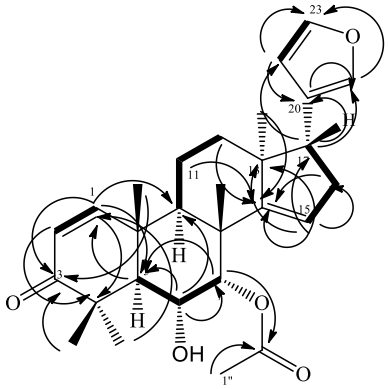

Figure 2. Selected HMBC and ^{1}H-^{1}H COSY correlations

The isolated compound's activity test was determined by testing their cytotoxic properties against murine leukaemia P-388 cells using the Alley method and obtained an IC_{50} value of 64.5 g/mL. This value indicates that nimonol activity is not active against P-388 leukaemia cells.

4. CONCLUSIONS

A limonoid compound, Nimonol, has been found in the seeds of *Chisocheton macrophyllus* (Meliaceae). The chemical structure of nimonol was determined by using spectroscopic data and comparing its structure with spectral data from previous studies. This nimonol compound was first discovered in *C. macrophyllus* seeds, but its activity against murine leukaemia P-388 cells was classified as inactive.

ACKNOWLEDGMENTS

This research was supported by the Directorate General of Higher Education, Ministry of Education and Culture, Indonesia (PDUPT Grant, No. 2393/UN6.3.1/PT.00/2022, Nurlelasari) Higher Education and supported by Universitas Padjadjaran (Academic Leadership Grant, No. 2203/UN6.3.1/PT.00/2022 by Unang Supratman). To Sofa Fajriah from LIPI Serpong for the NMR measurements. Thanks to Professor Yoshihito Shiono at the Department of Bioresource Engineering,

Faculty of Agriculture, Yamagata University, Japan, for HR-ESI-TOFMS measurements. This research is also supported by the Bogor Botanical Gardens. Thank you for the plant samples that have been given.

REFERENCES

Badria, F. A., & Ahmed, M. H. (2018). Azadiradione: A Multi-Targets Compound with New Therapeutic Approach. *Asian Journal of Phytomedicine and Clinical Research*, *6*(3), 115-120.

Chan, K. Y., Mohamad K., Ooi A. J. A., Imiyabir, Z., & Chung, L. Y. (2012). Bioactivity-guided fractionation of the lipoxygenase and cyclooxygenase inhibiting constituents from *Chisocheton polyandrus* Merr. *Fitoterapia*, *83*, 961–967. https:// doi.org/10.1016/j.fitote.2012.04.018.

Desi, H., Nurlelasari, Madilah, Deni, D., Maharani, R., Mayanti, T., & Supratman, U. (2018). Evaluation of the Acute Toxicity of Two Extracts of Chisochetonmacrophyllus Seeds in Female Wistar Rats. *Research Journal of Chemistry and Environment, 22*(Special Issue I) January.

Fitriana, R., Nurlelasari, Darwati, Harneti, D., Maharani, R., Mayanti, T., & Supratman, U. (2021). A New Limonoid from the Seeds of *Chisocheton lasiocarpus* (Meliaceae). *ALCHEMY Jurnal Penelitian Kimia, 17*(2), 219-226. https://doi.org/10.20961/alchemy.17.2.44782.

Hidayat, A. T., Nurlelasari, Abdullah, F. F., Harneti, D., Maharani, R., Haikal, K., Supratman, U., & Azmi, M. N. (2018). A New Lignan Derivative, Lasiocarpone, from The Stembark of *Chisocheton iasiocarpus* (Meliaceae). *Oriental Journal of Chemistry, 34*(4), 1956-1960. http://dx.doi.org/10.13005/ojc/3404032

Inada, A., Somekawa, M., Murata, H., Nakanishi, T., Tokuda, H., Nishino, H., Iwashima, A., Darnaedi, D., & Murata, J. (1993). Phytochemical Studies on Meliaceous Plants. VIII. Structures and Inhibitory Effects on Epstein-Barr Virus Activation of Triterpenoids from Leaves of *Chisocheton macrophyllus* King. *Chemical and Pharmaceutical Bulletin*, *41*(3), 617–619. https://doi.org/10.1248/cpb.41.617

Irungu, B. N. (2014). Phytochemical Investigation of *Turraea Robusta*, *Turraea Nilotica* and *Ekebergia Capensis For* Antiplasmodial And Cytotoxic Compounds**.** A Thesis Submitted in Fulfilment of the Requirements for Award of the Degree of Doctor of Philosophy in Chemistry of the University of Nairobi.

Katja, D. G., Farabi, K., Nuraini, V. A., Nurlelasari, Hidayat, A. T., Mayanti, T., Harneti, D., & Supratman, S. (2016). A New 30-nor Trijugin-type Limonoid, Chisotrijugin, from the Bark of *Chisocheton cumingianus* (Meliaceae). *International Journal of Chemistry*, *8*(3), http://dx.doi.org/10.5539/ijc.v8n3p30.

Katja, D. G., Sonda, A. A., Huspa, D. H. P., Tri Mayanti, dan Unang Supratman,. 2015. 7-Hidroksi-6-Metoksi Kumarin (Skopoletin) Dari Kulit Batang Chisocheton Celebicus (Meliaceae). *JURNAL KIMIA, 9(2), 267-270.*

Laphookhieo, S., Maneerat, W., Koysomboon, S., Kiattansakul, R., Chantrapromma, K., & Syers, J. K. (2008). A novel limonoid from the seeds of *Chisocheton siamensis*. *Canadian Journal of Chemistry*, *86*(3), 205–208. https://doi.org/10.1139/v07-155.

Lin, M., Bi, X., Zhou, L., & Huang, J. (2022). Insecticidal Triterpenes in Meliaceae: Plant Species, Molecules, and Activities: Part II (*Cipadessa*, *Melia*). *Int.J.Mol.Sci.,23*(10), 5329. https://doi.org/10.3390/ijms23105329.

Lin, M., Yang, S., Huang, J., & Zhou, L. (2021). Insecticidal Triterpenes in Meliaceae: Plant Species, Molecules and Activities: Part I (*Aphanamixis-Chukrasia*). *Int. J. Mol. Sci*, *22*(24),13262. https://doi.org/10.3390/ijms222413262.

Najmuldeen, I. A., Hadi, A. H. A., Mohamad, K., Awang, K., Ketuly, K. A., Mukhtar, M. R., Taha, H., Nordin, N., Litaudon, M., Guéritte, F., Nugroho, A. E., & Morita, H. (2012). Chisomicines D and E, Two New Limonoids from *Chisocheton Ceramicus*. *HETEROCYCLES*, *84*(2), 1265-1270.

Nguyen, K. V., Ho, D. V., Nguyen, H. M., Do, T. T., Phan, K. V., Morita, H., Heinamaki, J., Raal, A., & Nguyen, H. T. (2020). Chiro-Inositol Derivatives from *Chisocheton paniculatus* Showing Inhibition of Nitric Oxide Production. *J. Nat. Prod.*

https://dx.doi.org/10.1021/acs.jnatprod.9b01239.

Nurlelasari, Amalya, T. P., Julaeha, E., Huspa, D. H. P., Maharani, R., Darwati, & Supratman, U. (2020). 7-hidroksi-6-metoksi Kumarin (Skopoletin) dari Ekstrak Metanol Kulit Batang *Chisocheton cumingianus* (Meliaceae). *Chimica et Natura Acta*, *8*(2), 68-71. https://doi.org/10.24198/cna.v8.n2.29563.

Nurlelasari, Ferdiana, N., Mauluddin, S., Harneti, D., Moektiwardoyo, M., Abdullah, R., Supratman, U., & Awang. K. (2015). Senyawa Damara-20,24-dien-3-on yang beraktivitas Sitotoksis terhadap Sel Mcf-7 dari Kulit Batang *Chisocheton marcrophyllus* (Meliaceae). *IJAS*, *5*(3), https://doi.org/10.24198/ijas.v5i3.15062.

Nurlelasari, Huspa, D. H. P., Mayanti, T., & Supratman, U. (2016). Senyawa Disobinin Yang Bersifat Antimalaria dari Biji Tumbuhan *Chisocheton macrophyllus* (Meliaceae). *Al-Kimia*, *4*(2), https://doi.org/10.24252/al-kimia.v4i2.1675

Nurlelasari, Katja, D. G., Harneti, D., Wardayo, M. M., Supratman, U., & Awang, K. (2017). Limonoids from the seeds of *Chisocheton macrophyllus*. *Chemistry of Natural Compounds*, *53*(1), 83-87. https://doi.org/10.1007/s10600-017-1916-4

Nurlelasari, Rahmayanti, I., Salam, S., Safari, A., Harneti, D., Maharani, R., Hidayat, A. T., Tanjung, M., Retnowati, R., Shiono, Y., & Supratman, U. (2021). A new havanensin-type limonoid from *Chisocheton macrophyllus*. *Appl Biol Chem*, *64*:35, https://doi.org/10.1186/s13765-021-00606-5.

Rahmayanti, I., Nurlelasari, Harneti, D., Maharani, R., Darwati, Shiono, Y., & Supratman, U. 2021. Two Limonoids from The Seeds of *Chisocheton macrophyllus* and Their Cytotoxic Activity Against MCF-7 Breast Cancer Cells. *Molekul*, https://doi.org/10.20884/1.jm.2021.16.2.708.

Shilpi, J. A., Saha, S., Chong, S.L., Nahar, L., Sarker, S. D., & Awang, K. (2016). Advances in Chemistry and Bioactivity of the Genus *Chisocheton* Blume. *Chemistry and Biodiversity*, *13*(5), 483–503. https://doi.org/10.1002/cbdv.201400373.

Supratman, U., Salam, S., Naibaho, W., Fajar, M., Nurlelasari, Harneti, D., Maharani, R., Hidayat, A. T., Lesmana, R., Nafiah, M. A., & Shiono, Y. (2020). New cytotoxic limonoids from the stem bark of *Chisocheton pentandrus* (Blanco) Merr. *Phytochemistry Letters*, *35*, 63-67. https://doi.org/10.1016/j.phytol.2019.11.002.

Yang, M. H., Wang, J. S., Luo, J. G., Wang, X. B., & Kong, L. Y. (2012). Four new triterpenoids from *Chisocheton paniculatus* and their anti-inflammatory activities. *Can. J. Chem*, *90*, 199-204. https://doi.org/10.1139/v11-147.