PAPER • OPEN ACCESS

Structure Elucidation of Betulinic Acid from *Sesbania grandiflora* Root

View the article online for updates and enhancements.

Structure Elucidation of Betulinic Acid from *Sesbania grandiflora* Root

Noviany[1,*]**, H Osman**[2]

[1] Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Lampung, Jl. Sumantri Brojonegoro no 1, Bandar Lampung, Indonesia
[2] School of Chemical Sciences, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia

email: noviany@fmipa.unila.ac.id [1,*], ohasnah@yahoo.com[2]

Abstract. Betulinic acid, a known triterpene compound that has been successfully isolated from the ethyl acetate extract of the root of *Sesbania grandiflora*. The structure elucidation of betulinic acid was performed using one- and two-dimensional nuclear magnetic resonance, ultraviolet and infrared spectroscopy, and fast atomic bombardment mass spectrometry as well as by comparing with the literature data. This compound was found for the first time from the Fabaceae family, in particular *Sesbania grandiflora.*

Keywords: betulinic acid, *Sesbania grandiflora,* triterpene, structure elucidation

1.   Introduction

The term "natural products' is generally used to describe a broad collection of the chemical compounds or substances produced by a living organism found in nature, including plants, animals, marine, and microorganisms [1]. Natural products have been exploited by humans as medicine, flavor, poison, food, dye, and many other uses [2]. In recent years, there has been growing interest in the therapeutic use of natural products, particularly those derived from plants [3,4].

Plants produce a wide variety of so-called secondary metabolites that play an important role in producing the medicinal properties and in the survival of the plant in its ecosystem. Each plant species has its own specific set of secondary metabolites that is usually unique in its structural features [5]. The well-known classes secondary metabolites which displayed medicinal properties are terpenoids, steroids, alkaloids, and phenolics [6].

In the past years, there has been a rapidly increasing interest in plant secondary metabolites. About more than 100,000 plant secondary metabolites are already known, however, only a small percentage of all the plant species have been studied for their phytopharmacological properties [7]. Fabaceae plants, particularly species in the Papilionoideae subfamily, have long been extensively investigated for their phytochemical and pharmacological potentials. Some secondary metabolites including alkaloids, non-protein amino acids, flavonoids, coumarins, phenolics, anthraquinones, terpenoids, and glycosides have been reported from this plant [8]. Flavonoids are mostly obtained in the subfamily of Papilionoideae. *Sesbania grandiflora* is a member of the Fabaceae, subfamily Papilionoideae, and tribe Robinieae which is native to tropical Asia including India, Malaysia, Indonesia, Myanmar, and the Philippines. In our continuous investigation on Indonesian *S.grandiflora* plant, we have published some isoflavonoid compounds isolated from the root and phenolic compounds from the stem barks of *S.grandiflora* along with their biological properties [9-13]. In the current study, we reported the betulinic acid isolated from *S.grandiflora* roots as the first triterpene type found in this plant. The structure determination of this compound is discussed in this paper.

2. Experimental

All chemicals and solvents used for the extraction and isolation of the isolated compound were reagent grade. TLC has been conducted on silica gel 60 GF$_{254}$ plates (Merck; 0.25 mm) and sprayed with the staining reagent Ce(SO$_4$)$_2$. Column chromatography (CC) was carried out using silica gel (Kieselgel 60, 70–230 mesh ASTM; Nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR) spectra were recorded in dimethyl sulfoxide-d_6 (DMSO-d_6) with tetramethylsilane as an internal standard on a Bruker Avance III 400 MHz spectrometer and ^{13}C cryogenic probe or a Bruker 75 MHz. High-resolution ESI mass spectrometry was performed in positive ion mode on a 6230 TOF mass spectrometer (Agilent Technologies, Santa Clara, CA). IR (KBr) spectra were recorded using a Perkin-Elmer system 2000 FT-IR spectrometer. UV spectra were recorded using a Perkin-Elmer Lambda 25 spectrometer.

2.2   Plant material

S. grandiflora roots were collected in September 2008 in Sidosari village, South Lampung, Indonesia. The specimen of plant (No. N-III) was identified at the Herbarium Bogoriense, LIPI Bogor, Indonesia.

2.3   Extraction and isolation

Freshly chopped roots (4.0 kg) were cleaned by rinsing under running tap water to remove soil and dirt. The roots were dried in an open space for three weeks and the air-dried roots were finely ground into powder form. The powdered air-dried of the roots (1.5 kg) were macerated with 90% aqueous methanol (MeOH) (3 × 5 L) for seven days. The extract solvent was reduced using a rotatory evaporator at temperature of 40°C, yielding a sticky dark-brown extract (7.9 g). Next, the extract obtained was partitioned with *n*-hexane to afford a yellowish-brown *n*-hexane-soluble fraction (1.9 g) and a dark-brown MeOH-soluble fraction (5.8 g). The MeOH-soluble fraction was suspended in water and partitioned sequentially with chloroform (CHCl$_3$) and ethyl acetate (EtOAc), yielding a brownish CHCl$_3$-soluble fraction (0.9 g), a dark-brownish EtOAc-soluble fraction (3.4 g), and a reddish-brown of the MeOH-soluble fraction (0.9 g), respectively. A white precipitate was obtained during the extraction time. Repeated crystallization from the MeOH-soluble fraction using the eluent of EtOAc-MeOH (1:4 v/v) (three times) afforded compound **1**. The identity of compound **1** was determined by IR, NMR, and MS as well as compared with those reported earlier [14,15].

2.3.1 Betulinic acid (1)

Compound **1** was afforded as a white amorphous powder (23.7 mg); 0.5x10^{-3} % w/w of fresh roots, m.p. 276-278°C, Lit. m.p. 283–285°C [15]; $[\alpha]_D^{20} = -45.6$ (c = 0.1, MeOH); UV (MeOH) λ$_{max}$ (log ε) 205 (3.82); IR (KBr) v_{max} cm^{-1} 3449, 1686, 1641.

3. Results and discussion

Betulinic acid (**1**) was isolated as a white powder. The UV spectrum of **1** in methanol displayed λ$_{max}$ (log ε) at 205 (3.82) nm indicated the absence of a conjugated system. The IR absorption of **1** displayed the presence of the hydroxyl group at 3449 cm^{-1}, unsaturated carbonyl group at 1686 cm^{-1}, and an olefinic group at 1641 cm^{-1}. The negative-ion mode FABMS of **1** (Figure 1) displayed a pseudomolecular ion signal [M-H]$^+$ at *m/z* 455.4 which indicated a molecular formula of C$_{30}$H$_{48}$O$_3$ (calculated for C$_{30}$H$_{48}$O$_3$, 456.3605). This molecular formula was in agreement with the ^{1}H and ^{13}C NMR data (Table 1). By referring to the index of hydrogen deficiency [16], the molecular formula suggested that **1** had an unsaturation index of seven. The ^{1}H and ^{13}C NMR data (Table 1) collectively exhibited that one C=O carboxylic acid and one C-C double bond accounted for two out of seven degrees of unsaturation. The remaining five degrees of unsaturation was thus required for the presence of four hexacyclics and one pentacyclic core in compound **1**.

The ^{1}H NMR spectrum of **1** (Figure 2) showed the presence of six tertiary methyl groups (δ_H 0.65 – 0.93 ppm, *s*), two exo-methylene protons (δ_H 4.56, *br s*; 4.69, *br s*, each 1H), one carbonylic proton (δ_H 2.91-2.98, *m*), and the other methine signals appeared at δ_H 0.62 – 1.54 ppm. Similarly, the ^{13}C NMR spectrum (Figure 3) revealed signals for twenty-nine carbons which were distinguished as six methyls, eleven methylenes (ten aliphatic and one vinylic), six methines, and seven quaternary carbons (five aliphatics, one vinylic and one carbonyl). The types of carbon patterns were determined based on the DEPT experiments (90° and 135° sequence pulses at 25°C) (Figure 4a).
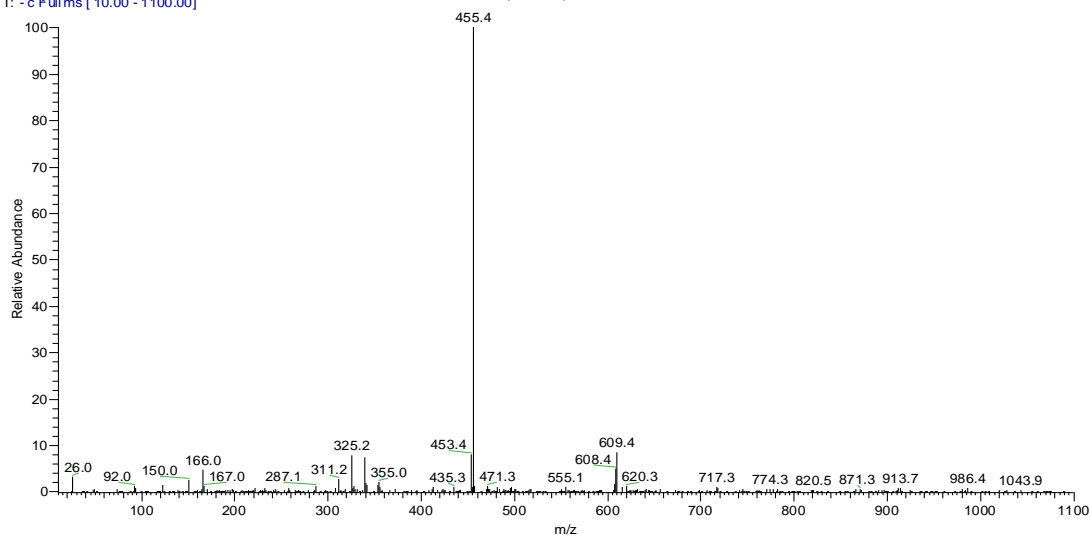
Figure 1. FABMS spectrum of **1** (negative-ion mode)

Table 1.   Comparison of ^{1}H NMR and ^{13}C NMR spectral data of compound **1**
and those of betulinic acid reported by Mutai *et al.* [14]

| No | ^{1}H NMR; δ_H (ppm); multiplicity; *J* (Hz) | | ^{13}C NMR; δ_C (ppm) | |
	1[a]	**Betulinic Acid[c]**	**1[b]**	**Betulinic Acid[d]**
1a	1.52, *dd* (11.2; 9.4)	0.86, *m*	39.1	38.7
1b	1.57-1.58, *m*	1.64, *m*		
2a	1.43-1.45, *m*	1.52, *m*	28.0	27.4
2b		1.61, *m*		
3	2.95-2.98, *m*	3.17, *m*	77.7	78.9
4			39.4	38.8
5	0.61-0.63, *m*	0.66, *m*	55.8	55.3
6a	1.48-1.50, *m*	1.50, *m*	18.8	18.2
6b	1.31-1.33, *m*			
7a	1.31-1.33, *m*	1.35, *m*	34.8	34.2
7b	1.45-1.47, *m*	1.40, *m*		
8			40.9	40.7
9	1.58-1.60, *m*	1.25, *m*	49.5	50.5
10			37.6	37.2
11a	1.23, *br s*	1.37, *m*	21.3	20.8
11b				
12a	0.97-0.99, *m*	1.04, *m*	25.9	25.5
12b	1.63-1.65, *m*	1.68, *m*		
13	2.21-2.24, *m*	2.20, *m*	38.5	38.3
14			42.9	42.4
15a	1.171.19, *m*	1.14, *m*	30.1	29.6
15b	1.23-1.25, *m*	1.49, *m*		
16a	1.36-1.38, *m*	1.27, *m*	32.6	32.1
16b	2.13, *dd* (11.3; 9)			
17			56.3	56.2
18	1.52-1.54, *m*	1.61, *m*	50.8	49.2
19	2.91-2.94, *m*	3.00, *m*	47.5	46.9
20			151.2	150.4
21a	1.77, *br d* (6.9 & 7.5)	1.99, *m*	31.0	30.5
21b	1.36-1.38, *m*	1.44, *m*		
22a	1.83, *br d* (6.9 & 4.3)	1.96, *m*	37.2	37.0
22b	1.42-1.44, *m*	1.55, *m*		
23	0.87, *s*	0.95, *s*	28.9	28.0

	a	c	b	d
24	0.65, s	0.73, s	16.8	15.3
25	0.76, s	0.80, s	16.6	16.1
26	0.93, s	0.91, s	15.3	16.0
27	0.87, s	0.96, s	16.6	14.7
28			178.1	181.4
29	4.56, br s ; 4.69, br s	4.58, s; 4.71, s	110.4	109.7
30	1.64, s	1.67, s	19.8	19.3
OH	4.26, br s	0.86, m	39.1	38.7

[a] 400 MHz, DMSO-d_6 ; [b] 75 MHz, DMSO-d_6.
[c] 400 MHz, CDCl$_3$; [d] 50.3 MHz, CDCl$_3$ [14].

The determination of the methyl group signals and the remaining ^{1}H and ^{13}C signals were performed through analyses of the COSY, HMQC, and HMBC experiments (Figure 4b-d). The results indicated that compound **1** is a lupane-type triterpene [14]. Based on its spectroscopy data (1D and 2D-NMR) and also by comparing its physicochemical, spectroscopic and mass spectrometric data with literature values [14,15], compound **1** was readily identified as a 3β-hydroxy-lup-20(29)-en-(28)-oic acid or betulinic acid (Figure 5).
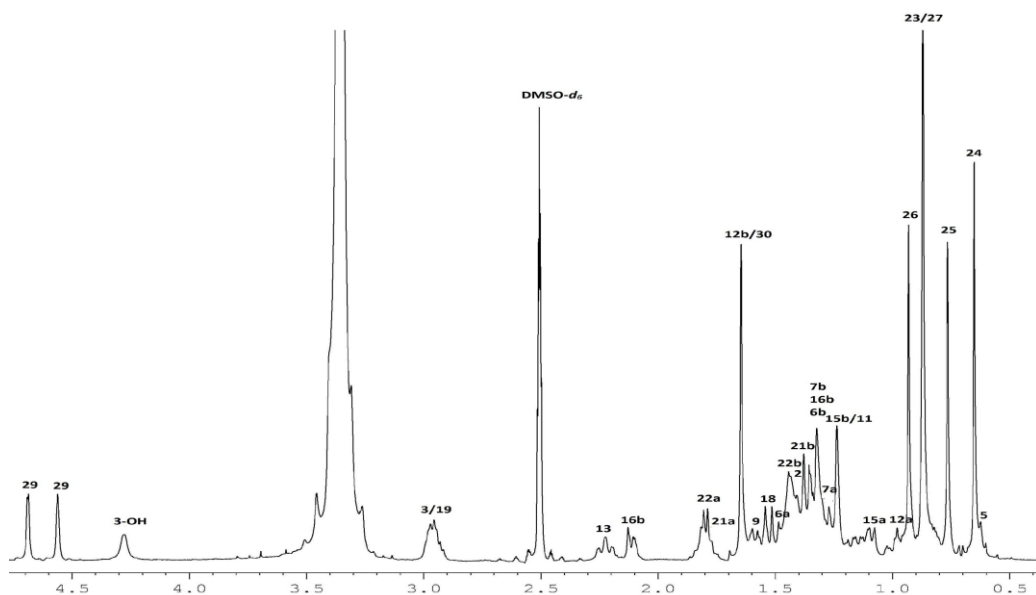

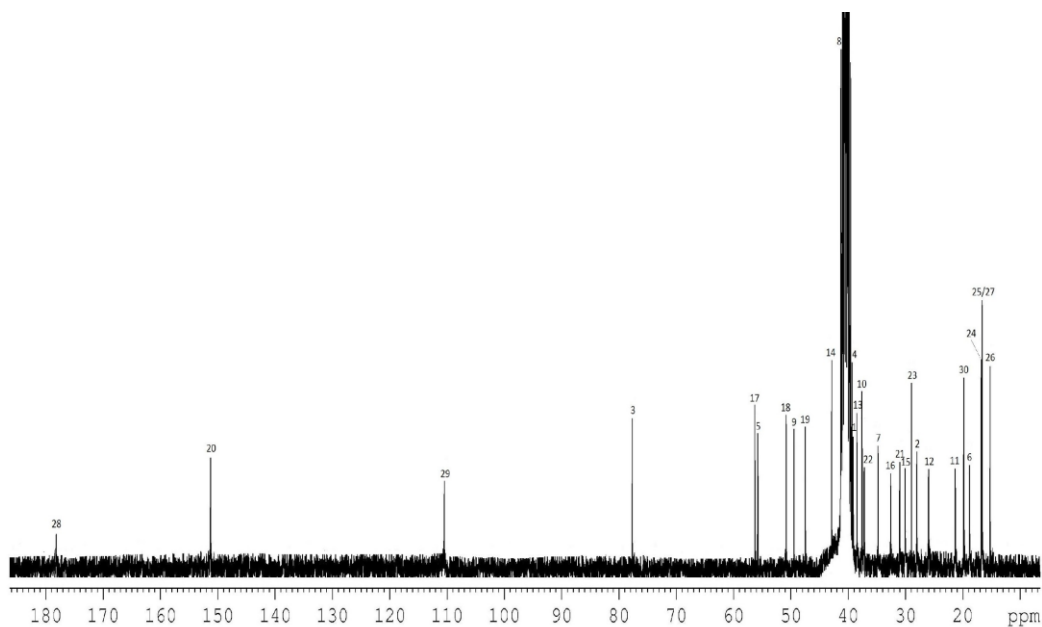**Figure 2.** ^{1}H NMR spectrum of **1** (400 MHz, DMSO-d_6)

Figure 3. ^{13}C NMR spectrum of **1** (75 MHz, DMSO-d_6)
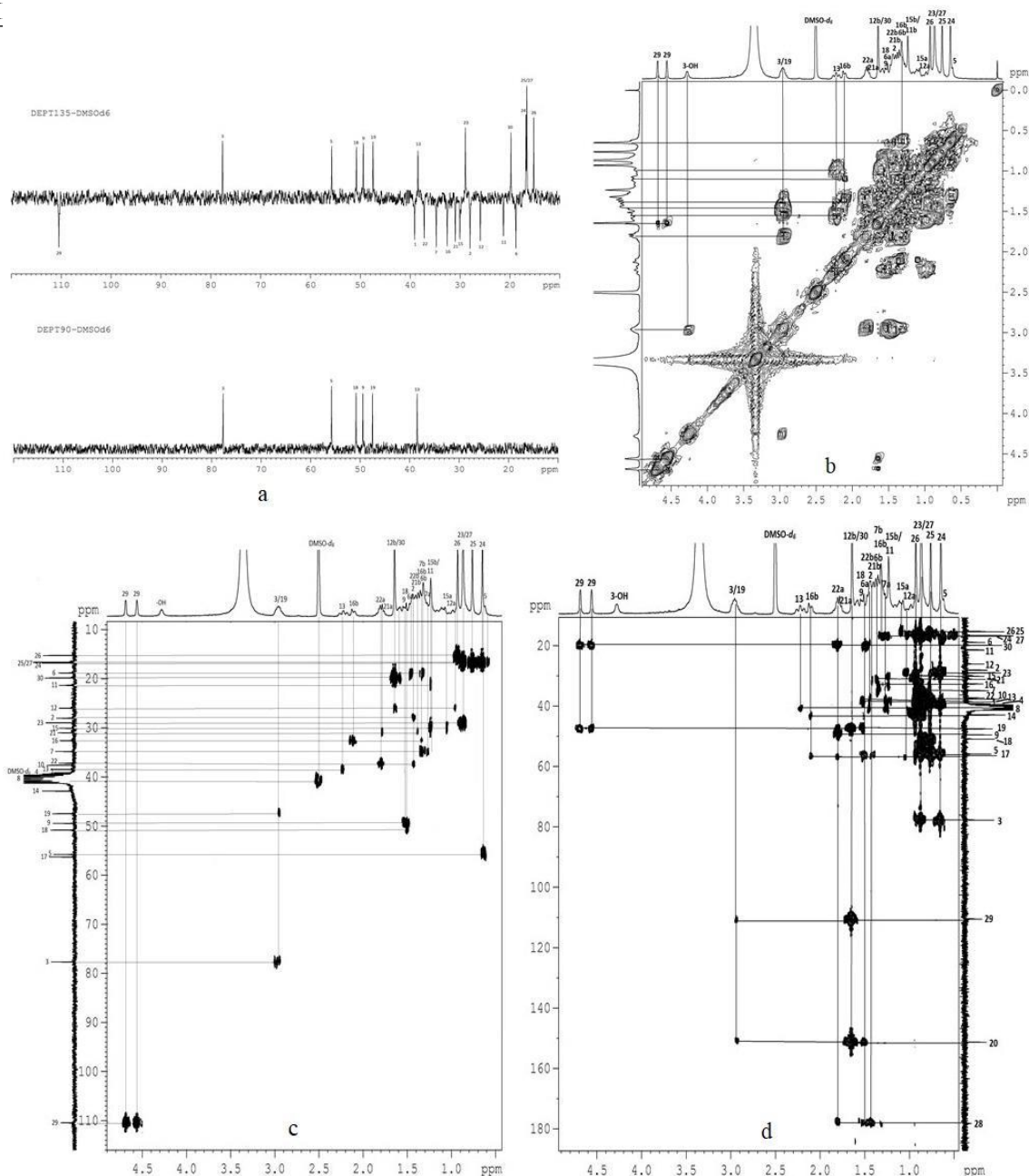
Figure 4. DEPT135 and DEPT90 NMR (a); ^{1}H-^{1}HCOSY (b); HMQC (c); HMBC spectra (d)
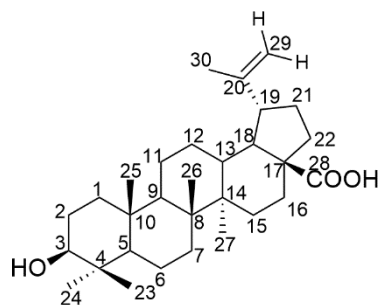

Figure 5. Structure of betulinic acid

4. Conclusions

In this study, a known triterpene constituent, betulinic acid was obtained from the EtOAc extract of *S. grandiflora* roots. Even though this compound is mostly found in certain tissue plants, however, it is first reported in the Fabaceae family. Since flavonoids and phenolics compounds are predominantly isolated from the Fabaceae family, therefore, this finding expands our understanding of the isolated compounds in the *Papilionoideae* genera belong to the Fabaceae family, particularly *S. grandiflora* species.

References
[1]   Bart H J 2011 *Extraction of natural products from plants – an introduction In S. P. Hans-Jörg Bart (Ed.), Industrial-scale natural products extraction* 1st ed (Germany: Wiley-VCH Verlag GmbH & Co) pp 1–25
[2]   Verpoorte R 2007 *Applications of plant metabolic engineering* (Netherlands: Springer) pp. 1–43
[3]   Chin Y W, Balunas M J, Chai H B, and Kinghorn A D 2006 Drug discovery from natural sources. *The AAPS J.* **8** 2, E239–E253
[4]   Newman D J, and Cragg G M 2012 Natural products as sources of new drugs over the 30 years from 1981 to 2010. *J. Nat. Prod.* **75** 311–335
[5]   Verpoorte R, Heijden R V D, Hoopen H J G T, and Memelink J 1999 Metabolic engineering of plant secondary metabolite pathways for the production of fine chemicals. *Biotech. Lett.* **21** 467–479
[6]   Hasan A 2007 *Phytochemical Analysis of Flavonoids* (Pakistan: Quaid-i-Azam University) pp. 1-3
[7]   Verpoorte R, Heijden R V D, and Memelink J 2000 Engineering the plant cell factory for secondary metabolite production. *Trans. Res.* **9** 323–343
[8]   Wink M, and Mohamed G I A 2003 Evolution of chemical defense traits in the Leguminosae: mapping of distribution patterns of secondary metabolites on a molecular phylogeny inferred from nucleotide sequences of the *rbc*L gene. *Biochem. Syst. Ecol.* **31** 8 897–917
[9]   Hasan N, Osman H, Mohamad S, Keng Chong W, Awang K, and Zahariluddin A S M 2012 The Chemical components of *Sesbania grandiflora* roots and their antituberculosis activity. *Pharmaceuticals*. **5** 882–889
[10]   Noviany, Osman H, Keng Chong W, Awang K, and Manshoor N 2012 Isolation and characterization of 1,1'-binaphthalene-2,2'-diol, a new biaryl natural product from *Sesbania grandiflora* root. *J. Bsc. Appl. Sci*. **8** 253–256
[11]   Noviany N, Nurhidayat A, Hadi S, Suhartati T, Aziz M, Purwitasari N, Subasman I 2018 Sesbagrandiflorain A and B: isolation of two new 2-arylbenzofurans from the stem bark of *Sesbania grandiflora*. *Nat. Prod. Res.* **32** 2558–2564
[12]   Noviany N, Samadi A, Yuliyan N, Hadi S, Aziz M, Purwitasari N, Mohamad S, Ismail N N, Gable K P, Mahmud T 2020 Structure characterization and biological activity of 2-arylbenzofurans from an Indonesian plant, *Sesbania grandiflora* (L.) Pers. *Phytochem. Lett.* **35** 211–215
[13]   Noviany N, Samadi A, Carpenter E L, Abugrain M E, Hadi S, Purwitasari N, Indra G, Indra A, Mahmud T. 2020 Structural revision of sesbagrandiflorains A and B, and synthesis and biological evaluation of 6-methoxy-2-arylbenzofuran derivatives. *J. Nat. Med.* DOI 10.1007/s11418-020-01445-2
[14]   Mutai C, Bii C, Vagias C, Abatis D, and Roussis V 2009 Antimicrobial activity of *Acacia mellifera* extracts and lupine triterpenes. *J. Ethnopharm.* **123** 143–148
[15]   Taralkar S V, and Chattopadhyay S 2012 A HPLC method for determination of ursolic acid and betulinic acids from their methanolic extracts of *Vitex Negundo* Linn. *J. Anal. Bioanal. Techniques.* **3** 3 pp 1–6
[16]   Breitmaier E 1999 *Structure Elucidation by NMR in Organic Chemistry* 2nd ed (Chichester: John Wiley) pp 42–178