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Phytochemical study of stem bark from *Alstonia spathulata* Bl. (Apocynaceae)

Kajian Fitokimia daripada Kulit Batang Pokok Alstonia spathulata Bl. (Apocynaceae)

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Abstract

In Malaysia, *Alstonia spathulata* Bl. is locally known as 'Pulai Basong' which distribute in peat forest. The separation of the chemical components from hexane and DCM crude extract of *Alstonia spathulata* was carried out using column chromatography (CC) and thin layer chromatography (TCL) techniques. A total of five known compound were isolated from the stem bark extracts and identified as: β-amyrin, β-amyrin acetate, lupeol acetate, stigmasterol and β-sitosterol. The elucidation structures of these compounds were established using 1D-NMR (^{1}H, ^{13}C and DEPT) and 2D-NMR (COSY, HSQC, HMBC) spectroscopic analysis, involving also comparison with data from the literature.

Keyword Phytochemical, *Alstonia spathulata*, bark extracts, hexane, chromatography, 1D-NMR and 2D-NMR

Abstrak

Di Malaysia, *Alstonia spathulata* B1 dikenali sebagai pokok 'Pulai Basong' yang banyak terdapat hidup di hutan paya. Proses pengasingan komponen kimia daripada ekstrak mentah heksana dan DCM *Alstonia spathulata* telah dijalankan menggunakan teknik turus kromatografi (CC) dan Kromatografi Lapisan Nipis (TLC). Sebanyak lima sebatian telah disaring dari ekstrak kulit batang dan dikenal pasti seperti berikut: β-amyrin, β-amyrin asetat, lupeol asetat, stigmasterol dan β-sitosterol. Penentuan struktur sebatian kimia diperolehi menggunakan kaedah analisis spektroskopi 1D-NMR (^{1}H, ^{13}C dan DEPT) dan 2D-NMR (COSY, HSQC, HMBC) serta perbandingan dengan data literatur.

Kata kunci Fitokimia, *Alstonia spathulata*, ekstrak kulit kayu, heksana, kromatografi, 1D-NMR dan 2D-NMR

INTRODUCTION

The genus *Alstonia* under tribe Alstonieae in the subfamily Rauvolfioideae of the family Apocynaceae is widely distributed in subtropical Africa, Central America, Southeast Asia, Polynesia and Australia, with most species found in the Malesian region (Webster's Online

Dictionary, 2012). *Alstonia spathulata* B1 is swamp dweller. The tree has a pagoda-like crown. The bark is smooth, grayish and produces abundant milky latex (Lim *et al*., 1998; Wiart, 2006). In Malaysia, *Alstonia spathulata* Bl. is locally known as 'Pulai Basong'. In Cambodia, Laos, Vietnam and Malaysia, its latex is applied externally to sores and diseased skin. The bark is used to lower fever and to expel worms from the intestine (Wiart, 2006). This study attempted to isolate and identify the chemical compounds from the bark of *Alstonia spathulata.* This paper further reported the isolation and characterization of five compounds, namely β-amyrin (**1**), β-amyrin acetate (**2**), lupeol acetate (**3**), stigmasterol (**4**) and β-sitosterol (**5**) shown in Figure 1.

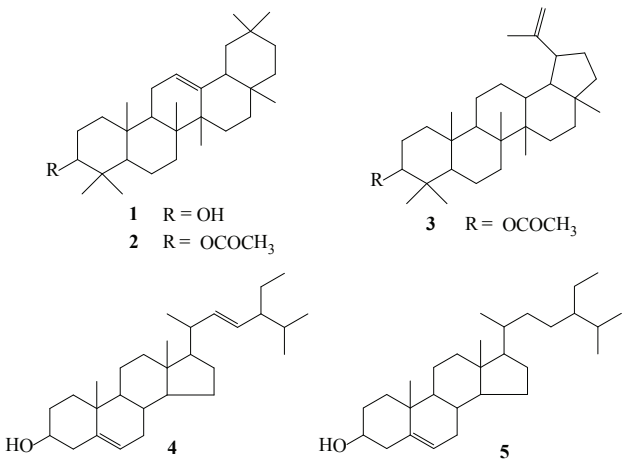

Figure 1 Compound isolated *Alstonia spathulata* B1

MATERIALS AND METHODS

General Methods

Nuclear Magnetic Resonance spectra (NMR) were recorded in deuterated chloroform ($CDCl_3$) on a BRUKER 600MHz and JEOL 500MHz. Chemical shifts (δ) were reported in ppm and coupling constants (*J*) in Hz. Mass spectra (MS) were determined by using GC-mass spectroscopy (GC-MS Agilent 5975 Series). The infrared spectra (IR) were recorded on a Nicolet 6700 FTIR spectrophotometer, with CH_2Cl_2 as dilution solvent of the sample. Column chromatography were prepared by using Silica Gel 60F, 70-230 mesh ASTM and, 230-400 mesh ASTM and Silica Gel 60 containing Gypsum F_{254} as stationary phase. Analytical thin layer chromatography (TLC) was performed on commercially precoated aluminium supported silica gel $60F_{254}$ TLC sheets.

Plant Material

The species selected for the current study is *Alstonia spathulata* Bl. was collected from Mersing, Johor and identified by the Phytochemical Group, Chemistry Department, Faculty Science, University of Malaya. The Voucher specimens were deposited at the Chemistry

Department, Faculty Science, University of Malaya, Kuala Lumpur, Malaysia and the Herbarium of the Forest Research Institute, Kepong, Malaysia.

Extraction and Isolation

Dried grounded bark of the *Alstonia spathulata* was extracted with hexane for 17 hours at room temperature. The extract was filter and dried by using rotary evaporator. Dried plants were rinsed with 25% ammonia solution for two hours and continued with acid-base extraction. This yielded organic layer and water layer (DCM extract residue). For the preliminary fractionation, hexane crude extract and DCM extract residue were subjected to column chromatography over silica gel and eluted with different combinations of solvent systems of hexane, dichloromethane and methanol mixture by increasing its polarity. Eluents were collected in fractions and were concentrated using rotary-evaporator. Each concentrated fractions was analysed by using aluminium supported TLC plate with a suitable solvent system and tested with 5% sulphuric acid. The fractions having spots with same retention factor (R_f) value were grouped together. Each grouped of fractions were treated separately by extensive column chromatography or preparative TLC for further purification process until single spot was showed on the TLC.

RESULTS AND DISCUSSION

Compound **1** was obtained as white amorphous. The IR spectrum showed stretching band for O-H (3360 cm^{-1}), olefin (1709 cm^{-1}) and C-O (1294 cm^{-1}). The mass spectrum of **1** showed a molecular ion peak at m/z 449 $[M+Na]^+$ corresponding to molecular formula $C_{30}H_{50}O$. From ^1H NMR spectrum (Table 1), methyl resonances of **1** were observed at δ 0.98, δ 0.77, δ 0.91, δ 0.95, δ 1.12, δ 0.81, δ 0.85 and δ 0.85 were corresponding to proton attached to C- 23, C-24, C-25, C-26, C-27, C-28, C-29 and C-30 respectively. A methine proton, signal at δ 3.20 (H-3, *dd*, *J*=11.0, 5.0 Hz) attached to a hydroxyl group and a signal δ 5.l6 (*t*, *J* = 6.8 Hz) was attributed to an olefinic proton at H-12.

Analysis of ^{13}C NMR spectrum (Table 2) revealed the presence of 30 carbons. There are seven quaternary carbons δ 39.9 (C-4), δ 38.9 (C-8), δ 37.0 (C-10), δ 145.3 (C-13), δ 41.8 (C-14), δ 32.8 (C-17), δ 31.2 (C-20); five methine carbons δ 79.1 (C-3), δ 55.3 (C-5), δ 47.7 (C-9), δ 121.8 (C-12), δ 47.2 (C-18); ten methylene carbons δ 38.7 (C-1), δ 27.3 (C-2), δ 18.5 (C-6), δ 32.7 (C-7), δ 23.6 (C-11), δ 26.2 (C-15), δ 27.0 (C-16), δ 46.9 (C-19), δ 34.8 (C-21), δ 37.2 (C-22); eight methyl carbons δ 28.2 (C-23), δ 15.7 (C-24), δ 15.6 (C-25), δ 16.9 (C-26), δ 26.1 (C-27), δ 28.5 (C-28), δ 33.5 (C-29), δ 23.8 (C-30). The alkene carbon appeared at δ 121.8 (C-12) and δ 145.3 (C-13) and hydroxyl carbon signal at δ 79.1 (C-3) were also observed. ^1H and ^{13}C NMR spectral data and mass spectrum were in agreement with those reported for ß-amyrin (Paz Lima, M. *et al*., 2004; Thanakijcharoenpath and Theanphong, 2007). Thus; compound **1** was identified as ß-amyrin.

Compound **2** was obtained as colourless needles (m.p.: 239.0-240.0 ºC). The IR spectrum displayed stretching band for ester carbonyl (1712cm^{-1}) and C-O (1264 cm^{-1}). The mass spectrum of **2** showed a molecular ion peak at m/z 468 $[M]^+$ corresponding to molecular formula $C_{32}H_{52}O_2$. The ^1H NMR spectrum (Table 1) of **2** exhibited for eight singlet methyl groups at δ 0.88, δ 0.96, δ 0.86, δ 0.97, δ 1.13, δ 0.83, δ 0.87, δ 0.87 were

Table 1 ^{1}H NMR [600 MHz, δ_H (*J*, Hz)] of compound **1**, **2**, **3**, **4** and [500 MHz, δ_H (*J*, Hz)] of **5** in $CDCl_3$

Position	1	2	3	4	5
1	1.61 (*m*)	1.63 (*m*)	0.99 (*m*)	1.07 (*m*)	1.08 (*m*)
	0.98 (*s*)	1.06 (*m*)		1.83 (*m*)	1.86 (*m*)
2	1.58 (*m*)	1.88 (*m*)	1.62 (*m*)	1.52 (*m*)	1.53 (*m*)
	0.77 (*m*)	1.60 (*m*)		1.83 (*m*)	1.86 (*m*)
3	3.20 (*dd*, *J*=11.0, 5.0Hz)	4.50 (*t*, 8.0 Hz)	4.47 (*dd*, 10.7 Hz and 5.5Hz)	3.52 (*m*)	3.55 (*m*)
4				2.23 (*m*)	2.29 (*m*)
				2.28 (*dd* 4.6Hz and 1.7Hz)	2.23 (*m*)
5	0.74 (*m*)	0.84 (*m*)	0.79 (*m*)		
6	1.53 (*m*)	1.53 (*m*)	1.50 (*m*)	5.35 (*br*, *s*)	5.35 (*m*)
	1.37 (*m*)	1.40 (*m*)	1.38 (*m*)		
7	1.50 (*m*)	1.52 (*m*)	1.49 (*m*)	1.46 (*m*)	1.49 (*m*)
	1.31 (*m*)	1.33 (*m*)	1.38 (*m*)	1.99 (*m*)	1.95 (*m*)
8				1.52 (*m*)	2.01 (*m*)
9	1.54 (*m*)	1.57 (*m*)	1.28 (*m*)	0.92 (*m*)	0.91 (*m*)
10					
11	1.85 (*m*)	1.80 (*m*)	1.39 (*m*)	1.52 (*m*)	1.44 (*m*)
		1.63 (*m*)	1.21 (*m*)		1.50 (*m*)
12	5.16 (*t*, 6.8Hz)	5.18 (*t*, 3.5Hz)	1.66 (*m*)	1.15 (*m*)	1.15 (*m*)
			1.05 (*m*)	2.02 (*m*)	2.02 (*m*)
13			1.65 (*m*)		
14				0.99 (*m*)	1.00 (*m*)
15	1.74 (*m*)	1.76 (*m*)	1.67 (*m*)	1.02 (*m*)	1.59 (*m*)
	0.95 (*m*)	0.85 (*m*)	1.00 (*m*)	1.58 (*m*)	
16	1.97 (*m*)	1.99 (*m*)	1.47 (*m*)	1.25(*m*)	1.26 (*m*)
		0.79 (*m*)	1.36 (*m*)	1.68 (*m*)	1.83 (*m*)
17				1.07 (*m*)	1.08 (*m*)
18	1.94 (*m*)	1.93 (*d*, 4. Hz)	1.35 (*m*)	0.68 (*s*)	0.68 (*s*)
19	1.64 (*m*)	1.66 (*m*)	2.40 (*m*)	1.00 (*s*)	1.01 (*s*)
	1.00 (*m*)	1.02 (*m*)			
20				2.02 (*m*)	1.35 (*m*)
21	1.31 (*m*)	1.08 (*m*)	1.91 (*m*)	1.18 (*d*, 5.7Hz)	0.92 (*d*,7.0Hz)
	1.10 (*m*)	1.33 (*m*)	1.33 (*m*)		
22	1.38 (*m*)	1.42 (*m*)	1.38 (*m*)	5.14 (*dd*, 15.5 Hz and 8.6 Hz)	1.03 (*m*)
	1.21 (*m*)	1.20 (*m*)	1.18 (*m*)		1.33 (*m*)
23	0.98 (*s*)	0.88 (*s*)	0.84 (*s*)	5.00 (*dd*, 15.5 Hz and 9.2Hz)	1.16 (*m*)
24	0.77 (*s*)	0.96 (*s*)	0.83 (*s*)	1.52 (*m*)	0.91 (*m*)
25	0.91 (*s*)	0.86 (*s*)	0.85 (*s*)	1.50 (*m*)	1.67 (*m*)
26	0.95 (*s*)	0.97 (*s*)	1.03 (*s*)	0.85 (*d*, 4.1Hz)	0.84 (*d*,6.90Hz)
27	1.12 (*s*)	1.13 (*s*)	0.94 (*s*)	0.79 (*d*, 6.8Hz)	0.81 (*d*,6.85Hz)
28	0.81 (*s*)	0.83 (*s*)	0.78 (*s*)	1.00 (*m*)	1.25 (*m*)
29	0.85 (*s*)	0.87 (*s*)	4.69 (*br*, *s*)	0.80 (*t*, 6.9Hz)	0.85 (*t*,7.45Hz)
			4.57 (*br*, s)		
30	0.85 (*s*)	0.87 (*s*)	1.68 (*s*)	-	
31	-			-	
32	-	2.05 (*s*)	2.05 (*s*)	-	

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Table 2 ^{13}C NMR [150 MHz, δ_H (*J*, Hz)] of compound **1**, **2**, **3**, **4** and [125 MHz, δ_H (*J*, Hz)] of **5** in $CDCl_3$

Position	1	2	3	4	5
1	38.7	38.3	38.4	37.3	37.2
2	27.3	23.5	23.7	31.7	31.6
3	79.1	81.0	81.0	71.9	71.8
4	39.9	39.8	37.8	42.4	42.3
5	55.3	55.2	55.4	140.8	140.7
6	18.5	18.3	18.2	121.8	121.7
7	32.7	32.6	34.2	31.9	31.9
8	38.9	37.7	40.9	31.9	31.8
9	47.7	47.6	50.3	50.2	50.1
10	37.0	36.8	37.1	36.6	36.5
11	23.6	23.6	20.9	21.2	21.1
12	121.8	121.6	25.1	39.8	39.7
13	145.3	145.2	38.0	42.3	42.3
14	41.8	41.7	42.8	56.8	56.7
15	26.2	26.1	27.4	24.4	24.3
16	27.0	26.9	35.6	29.0	28.2
17	32.6	32.5	43.0	56.0	56.0
18	47.2	47.2	48.3	12.0	11.8
19	46.9	46.8	48.0	19.5	19.4
20	31.2	31.1	151.0	40.6	36.1
21	34.8	34.7	29.8	21.2	18.8
22	37.2	37.1	40.0	138.3	33.9
23	28.2	28.0	28.0	129.3	26.0
24	15.7	15.6	16.5	51.3	45.8
25	15.6	16.7	16.2	32.0	29.1
26	16.9	16.8	16.0	21.2	19.8
27	26.1	26.0	14.5	19.1	19.0
28	28.5	28.4	18.0	25.5	23.0
29	33.5	33.4	109.4	12.1	12.0
30	23.8	23.7	19.3	-	-
31	-	171.1	171.1	-	-
32	-	21.4	21.3	-	-

corresponding to proton attached to C- 23, C-24, C-25, C-26, C-27, C-28, C-29 and C-30 respectively. It was identified as triterpene acetate due to the presence of an acetate methyl signal at δ 2.05 as a singlet. A methine proton, exhibited downfield shift signal at δ 4.50 (H-3, *t*, *J* = 8.0 Hz) which is attached to an acetoxyl group, compared with the corresponding proton in **1**. A signal δ 5.l8 (*t*, *J* = 3.5 Hz) was attributed to an olefinic proton at C-12.

Analysis of ^{13}C NMR spectrum (Table 2) showed the presence of 32 carbon atoms indicating a mono acetyl derivative. This attribution was corroborated by the presence of signals at δ 171.1 (C-31) which is characteristic of an ester carbon group. In addition, two

pairs of characteristic signals at δ 121.6 (C-12) and δ 145.2 (C-13) indicated it is olean type triterpene [4, 5], as well as presence of oxygenated carbons at δ 81.0 (C-3). There are seven quaternary carbons δ 39.8 (C-4), δ 37.7 (C-8), δ 36.8 (C-10), δ 145.2 (C-13), δ 41.7 (C-14), δ 32.5 (C-17), δ 31.0 (C-20); five methine carbons δ 81.0 (C-3), δ 55.2 (C-5), δ 47.6 (C-9), δ 121.6 (C-12), δ 47.2 (C-18); ten methylene carbons δ 38.3 (C-1), δ 23.5 (C-2), δ 18.3 (C-6), δ 32.7 (C-7), δ 23.6 (C-11), δ 26.1 (C-15), δ 26.9 (C-16), δ 46.8 (C-19), δ 34.7 (C-21), δ 37.1 (C-22); nine methyl carbons δ 28.0 (C-23), δ 15.6 (C-24), δ 16.7 (C-25), δ 16.8 (C-26), δ 26.0 (C-27), δ 28.4 (C-28), δ 33.4 (C-29), δ 23.7 (C-30), δ 21.4 (C-32) and the present of a carbonyl group at δ 171.1 (C-31). ^{1}H and ^{13}C NMR spectral data and mass spectrum were in agreement with those reported for ß-amyrin acetate (Ahmed *et al*., 2011; Segovia *et al*., 2011).. Thus; compound **2** was identified as ß-amyrin acetate.

Compound **3** was obtained as colourless amorphous. The IR spectrum showed stretching band for ester carbonyl (1712 cm^{-1}) and C-O (1263 cm^{-1}). The mass spectrum of **3** showed a molecular ion peak at m/z 468 $[M]^{+}$ corresponding to molecular formula $C_{32}H_{52}O_{2}$. The ^{1}H NMR spectrum (Table 1) of **3** exhibited six methyl singlets at δ 0.84, 0.83, 0.85, 1.03, 0.94, 0.78 and a vinylic methyl at 1.68 ppm which corresponding to proton attached to C- 23, C-24, C-25, C-26, C-27, C- 28 and C-30 respectively. It was identified as triterpene acetate due to the presence of an acetate methyl signal at δ 2.05 as a singlet. H-3 showed proton signal at δ 4.47 (*dd*, *J* = 10.7 Hz, 5.5 Hz). The presence of two exo-methylene at δ 4.57 and δ 4.69 (H-29) and one methyl signal at δ 1.68 (H-30) was readily recognized as lupane skeleton.

Analysis of ^{13}C NMR spectrum (Table 2) has indicated the presence of thirty two carbons. ^{13}C NMR data allowed the identification of ester linked methyl group (δ 21.3) and carbonyl functional group at δ 171.1 which is the characteristic of carboxylic acid. There are six quaternary carbons δ 37.8 (C-4), δ 40.9 (C-8), δ 37.1 (C-10), δ 42.8 (C-14), δ 43.0 (C-17), δ 151.0 (C-20); six methine carbons δ 81.0 (C-3), δ 55.4 (C-5), δ 50.3 (C-9), δ 38.0 (C-13), δ 48.3 (C-18), δ 48.0 (C-19); eleven methylene carbons δ 38.4 (C-1), δ 23.7 (C-2), δ 18.0 (C-6), δ 34.2 (C-7), δ 20.9 (C-11), δ 25.1 (C-12), δ 27.4 (C-15), δ 35.6 (C-16), δ 29.8 (C-21), δ 40.0 (C-22), δ 109.4 (C-29); eight methyl carbons δ 28.0 (C-23), δ 16.5 (C-24), δ 16.2 (C-25), δ 16.0 (C-26), δ 14.5 (C-27), δ 18.2 (C-28), δ 19.3 (C-30), δ 21.3 (C-32) and the present of a carbonyl group at δ 171.1 (C-31). The alkene carbon appeared at δ 151.0 (C-20) and δ 109.4 (C-29). The identified of **3** as lupeol acetate was confirmed comparison of these data with publish values of (Jamal and Ammar, 2009; Ajithabai, *et al*., 2011). Thus, compound **3** was identified as lupeol acetate.

Compound 4 was obtained as colourless needles (m.p: 151.0 - 152.0˚C). The mass spectral data of **4** gave a molecular formula $C_{29}H_{42}O$, m/z 413$[M+H]^{+}$. The IR spectrum displayed stretching band for O-H (3300 cm^{-1}), olefin (1711 cm^{-1}) and C-O (1263 cm^{-1}) (Figure 6). ^{1}H NMR spectrum (Table 2 and figure 4) consisted of two methyl singlets at δ 0.68 (H-18) and δ 1.00 (H-19), methyl doublets at δ 1.18 (H-21), δ 0.85 (*d*, *J* = 4.1 Hz, H-26) and δ 0.79 (*d*, *J* = 6.8Hz, H-27) and a methyl tripet at δ 0.80 (H-29). A hydroxyl methine proton resonated at δ 3.52 as a multiplet corresponding to proton (H-3). Three olefinic protons in which the last two are of trans configuration were observed at δ 5.35, δ 5.14 (*dd*, *J* = 15.5 Hz and 8.6 Hz) and δ 5.00 (*dd*, *J* = 15.5 Hz and 9.2 Hz) attributable to H-6, H-22 and H-23 respectively.

^{13}C NMR spectrum (Table 2) of compound **4** exhibited twenty nine signals. Signal for six methyl carbons C-18, C-19, C-21, C-26, C-27 and C-29 could be seen in the most upfield

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region of the NMR spectrum at δ 12.0, δ 19.5, δ 21.2, δ 21.2 δ 19.1 and δ 12.1. The position of three quaternary carbons appreared at δ 140.8 (C-5), δ 36.6 (C-10), δ 42.3 (C-13); nine methylene carbons: δ 37.3 (C-1), δ 31.7 (C-2), δ 42.4 (C-4), δ 31.9 (C-7), δ 21.2 (C-11), δ 39.8 (C-12), δ 24.4 (C-15), δ 29.0 (C-16), δ 29.8 (C-28); and eleven methine carbons: δ 71.9 (C-3), δ 121.8 (C-6), δ 31.9 (C-8), δ 50.2 (C-9), δ 56.8 (C-14), δ 56.0 (C-17), δ 40.6 (C-20), δ 138.4 (C-22), δ 129.3 (C-23), δ 51.3 (C-24), δ 45.9 (C-25). Signal at δ 140.8, δ 121.8, δ 138.3 and δ 129.3 were observed for four olefinic carbons, corresponding to C-5, C-6, C-22 and C-23 respectively. A deshielded signal of δ 71.9 confirmed the presence of hydroxyl group attached to C-3. This compound was found to be a C-5 unsaturated modified tetracyclic triterpene, which showed only 29 carbons instead of 30 carbons for tetracyclic triterpenes. Comparison of these ^{1}H and ^{13}C NMR data with literature (Jamal *et al*., 2009; Pateh *et al*., 2009) showed that compund **4** is stigmasterol.

Compound **5** was obtained as white amorphous. On subjection to IR spectroscopic analysis, the observed absorption bands are 3347.1 cm^{-1} that is characteristic of O-H strecthing. Absorption at 1642.9 cm^{-1} is due to olefinic strecthing (C=C) and absorption at 1032.3 cm^{-1} attributed to C-O strecthing. The mass spectrum data showed the molecular ion peak at m/z 414 that correspond to the molecular formula $C_{29}H_{50}O$.

The ^{1}H NMR has revealed the existence of signals for two angular methyl proton at δ 0.68 and δ 1.01; three methyl doublets at δ 0.92 (*d*, *J* = 7 Hz), δ 0.84 (*d*, *J* = 6.90 Hz), δ 0.81 (*d*, *J* = 6.85 Hz) and a methyl tripet at δ 0.85 (*t*, *J* = 7.45 Hz) which corresponding to C-18, C-19, C-21, C-26, C-27 and C-29 proton respectively. A hydroxyl methine proton appear at δ 3.55 as a multiplet corresponding to proton H-3. The typical signal for the olefinic proton H-6 of the steroidal skeleton was evident as a multiplet at δ 5.35 intergrating for one proton.

Total of 29 carbon atoms was revealed from the ^{13}C NMR spectrum. This compound is having six methyl carbons: δ 12.0 (C-18), δ 19.5 (C-19), δ 18.9 (C-21), δ 19.9 (C-26), δ 19.0 (C-27) and δ 12.1 (C-29); eleven methylene carbons: δ 37.3 (C-1), 31.7 (C-2), δ 42.4 (C-4), δ 32.0 (C-7), δ 21.2 (C-11), δ 39.9 (C-12), δ 24.4 (C-15), δ 28.3 (C-16), δ 34.0 (C-22), δ 26.1 (C-23) and 23.1 (C-28); nine methine carbons: δ 71.9 (C-3), δ 121.8 (C-6), δ 32.0 (C-8), δ 50.2 (C-9), δ 56.8 (C-14), δ 56.1 (C-17), δ 36.2 (C-20), δ 45.9 (C-24) and δ 29.2 (C-25) and three quaternary carbons: δ 140.8 (C-5), 36.6 (C-10), and 42.4 (C-13). The ^{13}C NMR has shown recognizable signals δ 140.8 and δ 121.8ppm, which are assigned C-5 and C-6 respectively. The δ value at 71.9ppm is due to presence of hydroxy group at C-3. Signals at δ 12.0 and δ 19.5 corresponds to angular carbon atom C-18 and C-19. The above spectral data and comparison of the ^{13}C NMR signal with those described in the literatures (Agrawal *et al*., 1985; Pretsch *et al*., 2000; Mouffok *et al*., 2012) showed the compound **5** to be the ß-sitosterol. Compound **5** have same skeleton with **4**, both of the compound are steroid. The only difference between the two compound is the presence of C22=C23 double bond in **4** and C22-C23 single bond in **5**.

CONCLUSIONS

The research work through a systematic chemical investigation has determined and identified for the first time the presence of β-amyrin **1**, β-amyrin acetate **2**, lupeol acetate **3**, stigmasterol **4** and β-sitosterol **5**. from the bark of *Alstonia spathulata.* The work was

carried out by means of various physical (solvent extraction and column chromatography) and spectral techniques. The assignment will be further supported by 2D-NMR (COSY, HSQC, and HMBC) spectroscopic analysis. The structures of the compounds were also elucidated by comparison with literature.

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