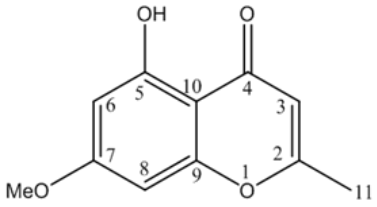
PHENOLIC COMPOUND, TRITERPENE AND STEROIDS FROM THE LEAVES AND BARK OF *DYSOXYLUM MACROCARPUM*

Ibrahim **A**. **N**ajmuldeen,[1] **K**amal **A**ziz **K**etuly[2], And **A**. **H**amid **A**. **H**adi[3]
[1]Department of Chemistry, Faculty of Science, University of Zakho, Zakho, Kurdistan Region, Iraq.
[2]Department of Biochemistry, Faculty of Medicine, University of Dohuk, Dohuk, Kurdistan Region, Iraq.
[3]Department of chemistry, Faculty of science, University Malaya, 50603 Kuala Lumpur, Malaysia.
(Accepted for publication: June 9, 2013)

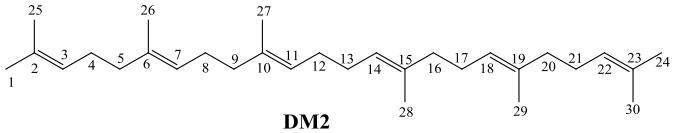
Abstract

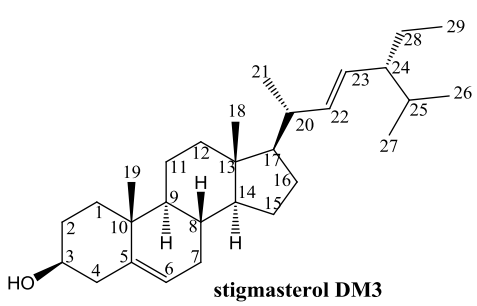
The bark and leaves of *Dysoxylum macrocarpum* (Meliaceace) yielded four compounds, two compounds from the leaves; 5-hydroxy-7-methoxy-2-methyl-4*H*-chromen-4-one (Eugenin) DM1, which was new as crystal and squalene DM2, while two more compounds were found from the bark; stigmasterol DM3 and sitosterol DM4.

The present work involves extraction, isolation and purification of compounds by using column chromatography followed by preparative TLC. Structural elucidation has been done through several spectroscopic methods, notably UV, IR, MS (HRMS, GCMS, and LCMS), 1D, 2D-NMR ^{1}H NMR, ^{13}CNMR, COSY, DEPT, HMQC, HMBC, and single crystal X-ray diffraction analysis.

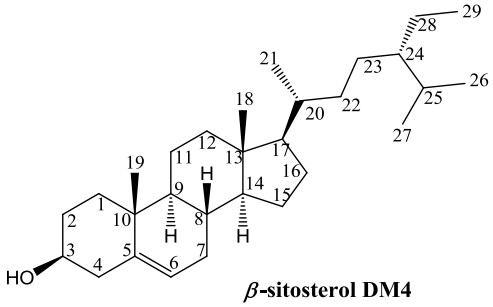


DM1


DM2



stigmasterol DM3



***β*-sitosterol DM4**

Key word: Meliaceace, *Dysoxylum macrocarpum*, Phenolic Compound , Triterpene, steroids.

Introduction

In continuing of our researches on the Meliaceae genera (Najmuldeen et al., 2008; Najmuldeen et al., 2010a; Najmuldeen et al., 2010c; Najmuldeen et al., 2011), we have studied the chemical constituents of the bark and leaves of *Dysoxylum macrocarpum* (Meliaceace). *Dysoxylum macrocarpum* is a big tree of 25 to 33 m tall and 1.5 m girth. Plank buttresses to 2 m tall and to 1 m out, Its bark smooth, grey-green, faintly hooped and finely lenticellate; inner bark cream, flecked orange, fibrous within; wood pale yellow. Leafy twigs stout with wide pith and conspicuous leaf scars and lenticels. Apical buds spike-like to 5 cm long. Leaves to 1 m long, paripinnate, subglabrous; leaflets in 3 or 4 pairs, to 30 cm long and with 13-18 veins on each side of midrib but other venation obscure. Inflorescence to 25 cm long, with spreading branches. Flowers foetid; petals 4; anthers 8 (9). Infructescence with massive axis to 8 mm diam., of 1-3 fruits, globose, 10 cm diam., bright orange-red, shallowly ridged, dehiscent; pericarp with white latex; mesocarp fleshy, orange-yellow; seeds 1-2 with dark brown (7) sarcotesta (Mabberly and Pannell, 1989).

From our literature review we have listed in Table 1 some selected chemicals of *Dysoxylum*

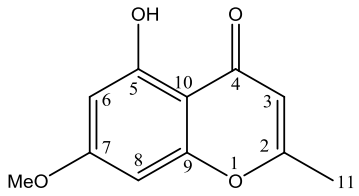
species, and no chemical investigation has been found on *Dysoxylum macrocarpum.*

Table1: Occurrence of some selected chemicals in species of *Dysoxylum*

No.	*Dysoxylum* pecies	Type of Compounds	Examples	Ref.
1	*Dysoxylum acutangulum*	triterpenoids	acutaxyline A	(Ismail et al., 2009)
2	*Dysoxylum binectariferum*	chromane alkaloids	rohitukine	(Bhat et al., 1986; Cui et al., 2008; Lakdawala et al., 1988; Mohanakumara et al., 2010; Naik et al., 1988; Yang et al., 2004)
3	*Dysoxylum cauliflorum*	triterpenoid	(20R)-23(24-25)abeo-20,24 -dihydroxydammaran-3-one	(Huang et al., 1999)
4	*Dysoxylum densiflorum*	degraded limonoids sesquiterpenoids limonoid acids	dysodensiol A dysodensiol D dysoxylumic acid A	(Xie et al., 2008a; Xie et al., 2008b)
5	*Dysoxylum hainanense*	diterpenoid triterpenoid	ent-18-acetoxy-8-(14)-pimarene-15S,16-diol67 3*β*,22S-dihydroxy-tirucalla-7,24-dien-23-one	(He et al., 2009; He et al., 2008a; He et al., 2008b; Luo et al., 2002a; Luo et al., 2002b; Luo et al., 2000a; Luo et al., 2000b; Luo et al., 2000c; Luo et al., 2001a; Luo et al., 2001b)
6	*Dysoxylum gillespie*	biflavonoid	rubustaflavone 4',7''-dimethyl ether	(He et al., 1996)
7	*Dysoxylum kuskusense*	diterpenoids	dysokusone A	(Duh et al., 2000a; Duh et al., 2000b; Fujioka et al., 1998a; Fujioka et al., 1998b)
8	*Dysoxylum macranthum*	triterpenoids	dymacrin B	(Mohamad et al., 1999)
9	*Dysoxylum malabaricum*	steroids	ergosta-5,24(24¢)-diene-3b,4b,20S-triol	(Govindachari et al., 1994; Govindachari et al., 1996; Govindachari et al., 1999; Hisham et al., 1996; Hisham et al., 2001)
10	*Dysoxylum muellerii*	protolimonoid	cabraleone	(Mulholland and Naidoo, 2000; Mulholland et al., 1996)
11	*Dysoxylum richii*	sulfuric comp. limonoids triterpenoids	dysoxysulfone dysoxylin richenone	(Aalbersberg and Singh, 1991; Jogia and Andersen, 1987; Jogia and Andersen, 1989; Jogia et al., 1989; Singh and Aalbersberg, 1992)
12	*Dysoxylum schiffneri*	sesquiterpenoids	schiffnerone	(Mulholland et al., 1998)
13	*Dysoxylum spectabile*	limonoids lriterpenoids	methyl ivorensate isopimara-8(14),15-diene	(Mulholland et al., 1999; Russell et al., 1994)

Results and discussion

5-Hydroxy-7-methoxy-2-methyl-4*H*-chromen-4-one (Eugenin) DM1



Compound **DM1** was isolated for the first time as white crystal m.p118-119 °C (Najmuldeen et al., 2010b). The UV spectrum showed λ_{max} 207, 232, 250, 256, 290 and 316 nm. The IR absorption showed a broad signal at ν_{max} 3434 cm^{-1} assignable to OH and a strong absorption at ν_{max} 1636 cm^{-1} which was characterized for a conjugated ketone. The LCMS spectrum showed a molecular positive ion peak $[M+H]^+$ at m/z 207 which consistent with the molecular formula $C_{11}H_{10}O_4$+H.

Furthermore, in the ^{1}H-NMR spectrum (Table 2, Figure 1) of **DM1** showed three singlet peaks two of them were sharp singlet at δ_H 2.34 and 3.84 due to methyl and methoxy protons , respectively, and one singlet at exact δ_H 6.00 indicated the proton of H-3. Two doublets at δ_H 6.30 and 6.33 indicated the presence of two protons in *meta* positions of H-6 and H-8,

respectively.

The ^{13}C-NMR and DEPT spectra (Table 2, Figure 2) of **DM1** exhibited 11 signals corresponding to 11 carbon resonances. Signal at δ_C 182.6 was ascribable to conjugated carbonyl carbon C-4. Furthermore five sp^2 quaternary carbons among them four were oxygenated (C-2, C-5, C-7, C-9) and appeared in more downfield in comparison with the fifth one C-10. In COSY spectrum no correlation was found. The structure has been further approved by HMQC (Table 2, Figure 3), HMBC (Figure 4), X-Ray (Figure 5), and LCMS (Figure 6). Based on the spectral data and comparison with literature (Tsui and Brown, 1996), compound **DM1** was assigned as 5-Hydroxy-7-methoxy-2-methyl-4*H*-chromen-4-one.

Table 2 : 1D (^{1}H and ^{13}C) and 2D (HMQC and HMBC) NMR spectral data of eugenin DM1 in $CDCl_3$

Position	δ_H (int.; mult.; J(Hz))	δ_C	HMQC	HMBC
2		166.9		
3	6.00 (1H, s)	108.8	H-C3	2, 4, 10, 11
4		182.6		
5		162.2		
6	6.30 (1H, d, 2.0)	98.0	H-C6	4, 5, 7, 8, 10
7		165.4		
8	6.33 (1H, d, 2.0)	92.5	H-C8	6, 7, 9, 10
9		158.2		
10		105.3		
11	2.34 (3H, s)	20.6	H_3-C11	2, 3
MeO	3.84 (3H, s)	55.8	H_3-MeO	7

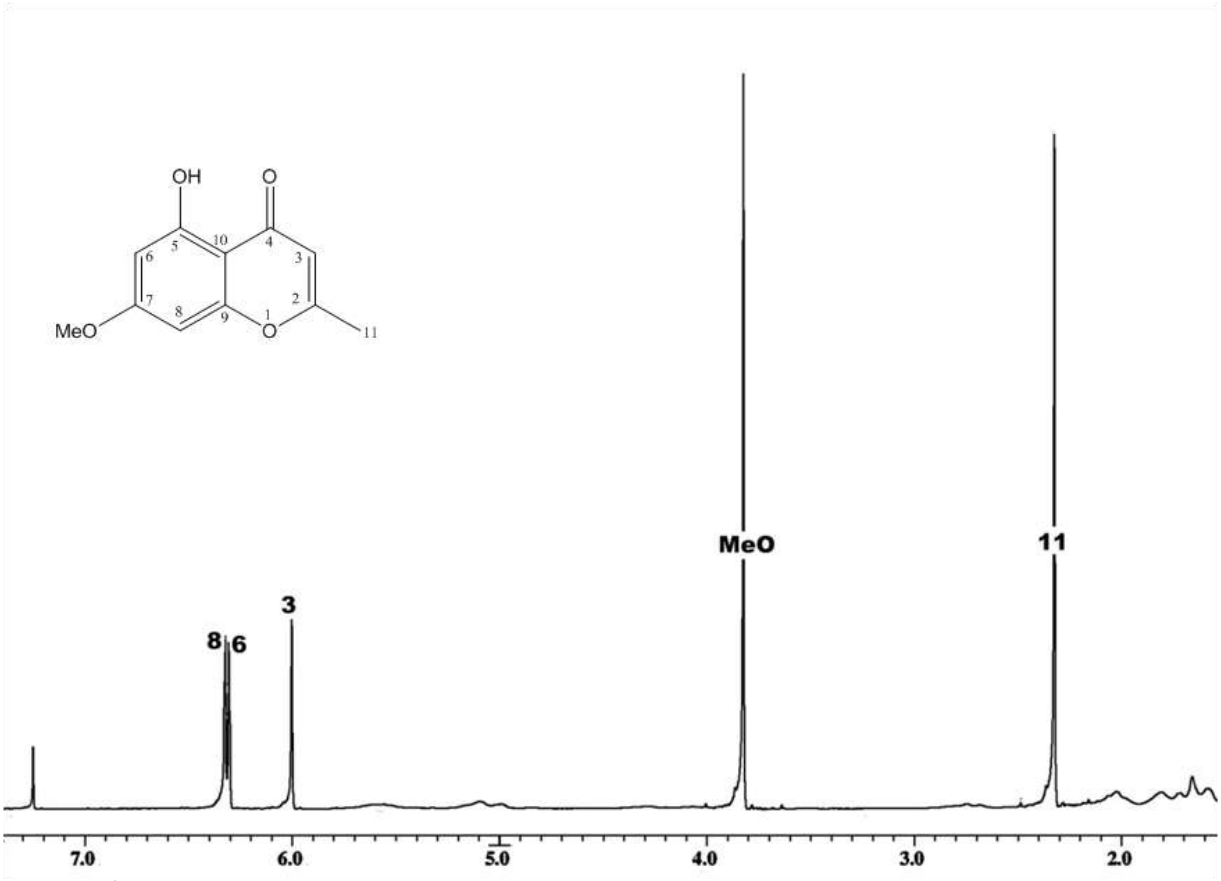


Figure 1: ^{1}H-NMR spectrum of Eugenin DM1

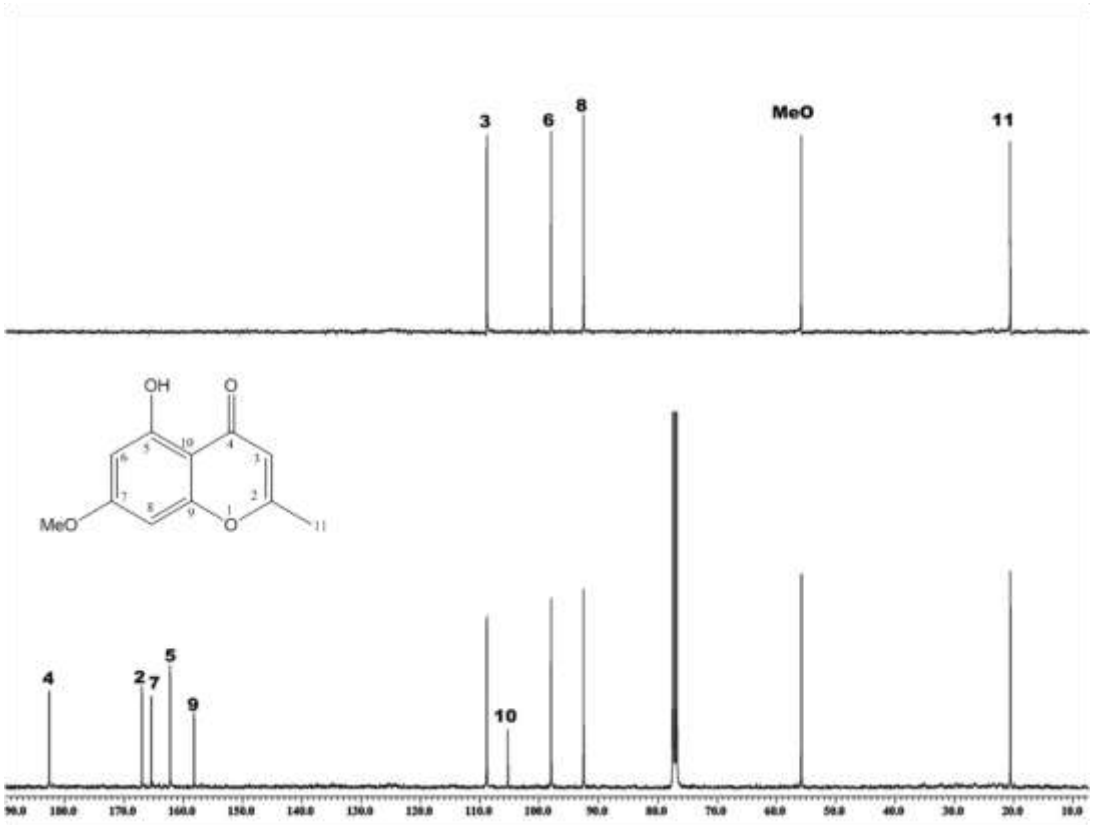


Figure 2: ^{13}C/DEPT-NMR specta of Eugenin DM1

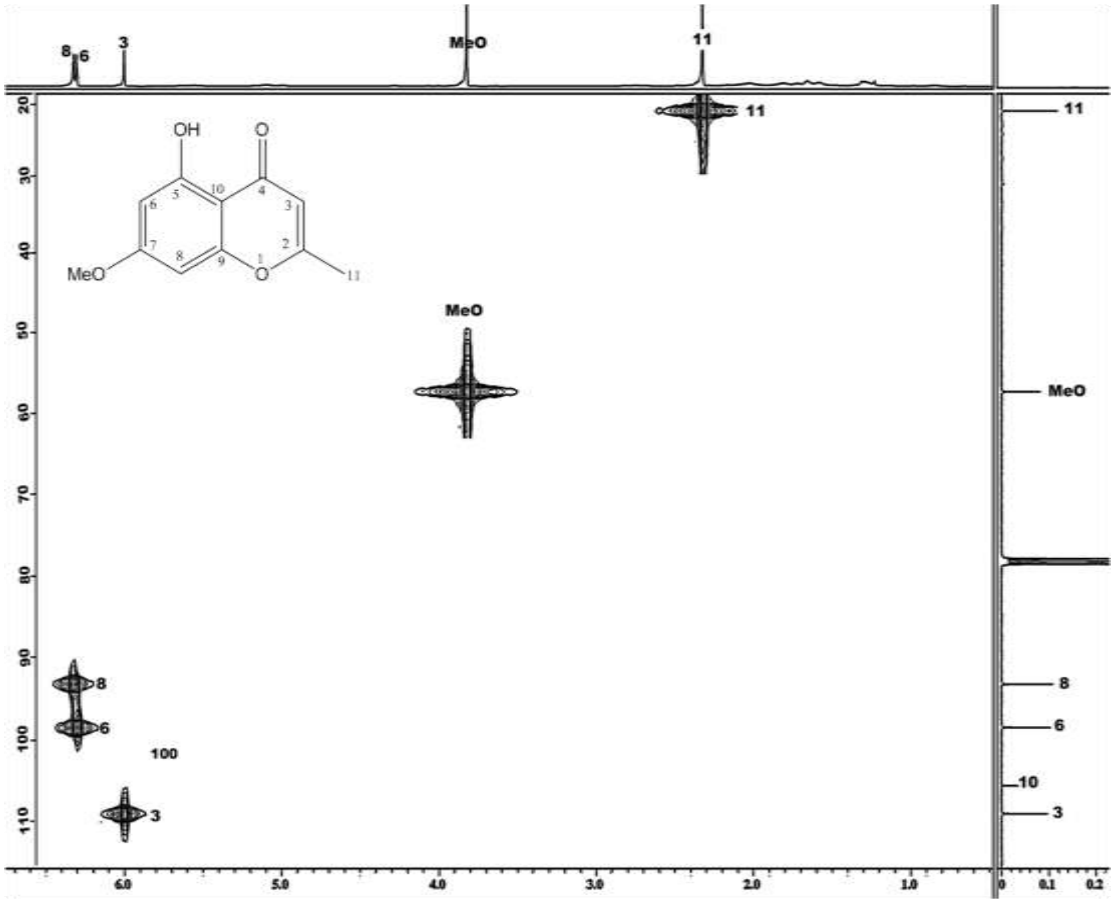


Figure 3: HMQC spectrum for Eugenin DM1

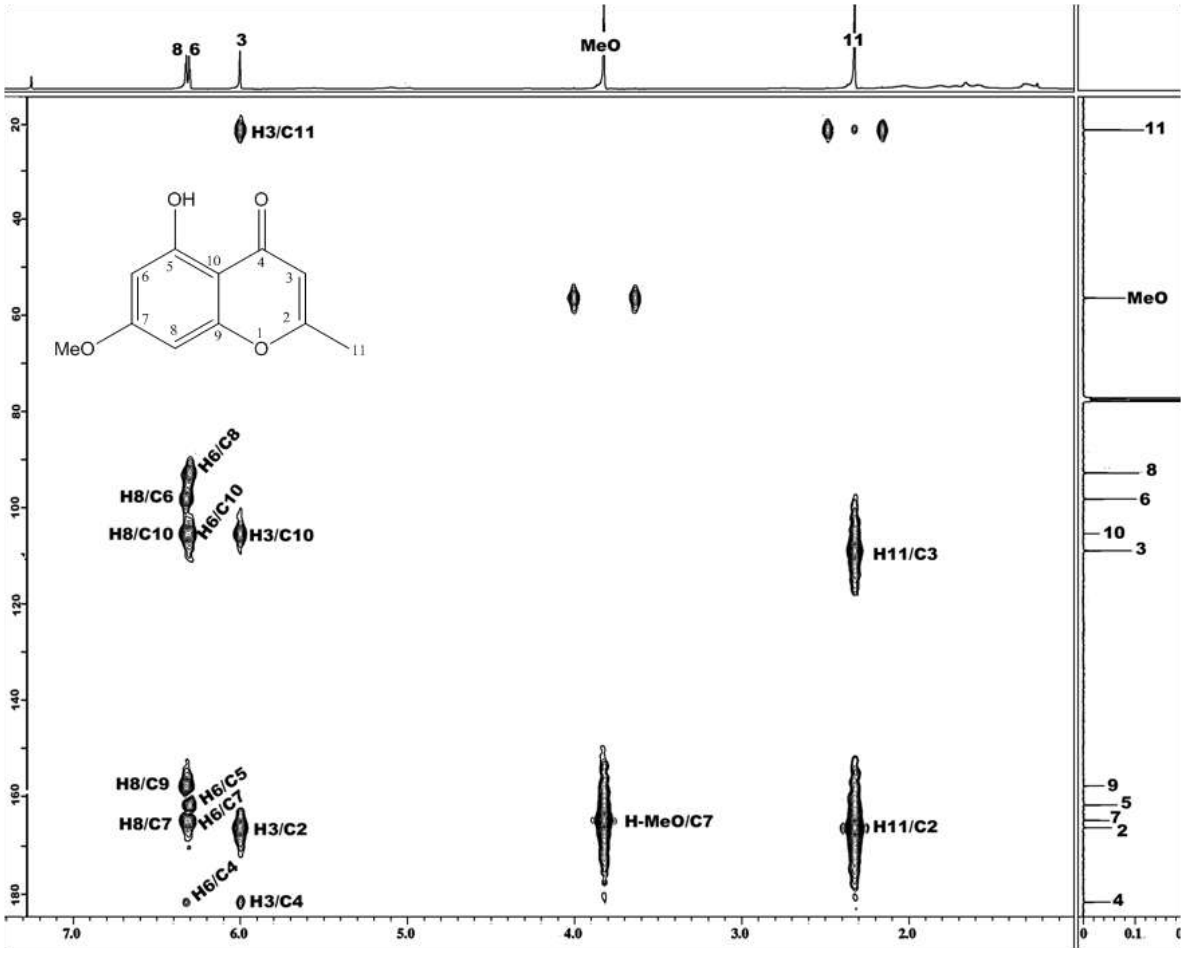
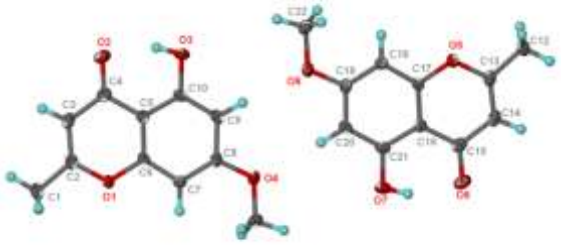


Figure 4: HMBC spectrum of Eugenin DM1

Note :- numbering of X-ray crystallographic structure is different from the numbering of normal



chemical structure.

Figure 5: X-Ray crystallographic structure of Eugenin DM1

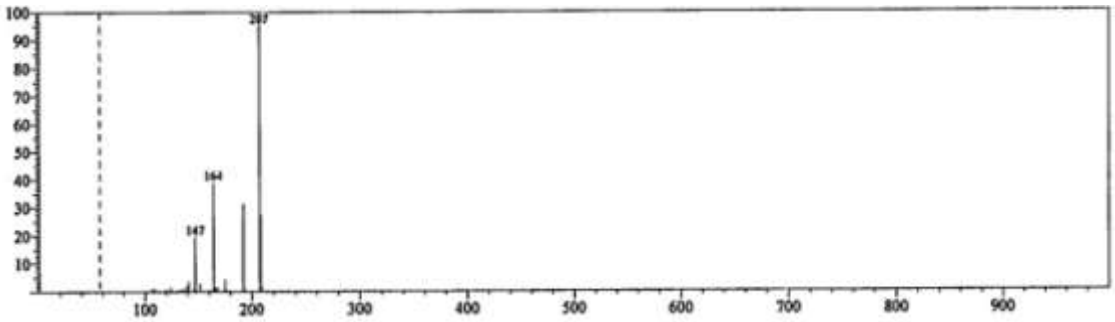
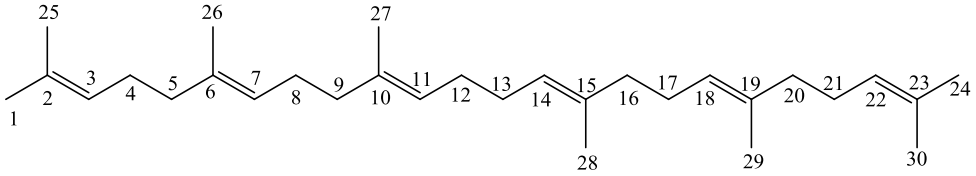


Figure 6: LCMS of Eugenin DM1

Squalene DM2



Compound **DM2** was isolated as yellow colored oil with b.p. 285°C. The UV spectrum showed λ_{max} at 344 nm. The IR spectrum showed signals at ν_{max} 2914 (C-H stretching), 2728, 1668 (alkene, non-conjugated), 1446 (alkane, CH_2), 1382 (alkane, CH_3), 1330, 1224, 1151, 1188, 964 (alkene, disubstituted trans), 835 (two adjacent hydrogen atoms). The GCMS spectrum showed the molecular ion mass $[M]^+$ at m/z 410 which consistent with the molecular formula $C_{30}H_{50}$.

The ^{1}H-NMR spectrum (Table 3, Figure 7) consists of four signal groups dispersed over the sp^3 and sp^2 chemical shift range. In the olefinic regions, six protons (H-3, H-7, H-1 1, H-14, H-18 and H-22) were found at δ_H 5.10, the signal groups of twenty protons (ten methylene) appeared at δ_H 2.05 belongs to H_2-4, H_2-5, H_2-8, H_2-9, H_2-12, H_2-13, H_2-16 H_2-17, H_2-20 and H_2-21. Meanwhile, a ^{1}H-NMR spectrum consisted a singlet at δ_H 1.66, that clearly belonged to H_3-1 and H_3-24, together with a broad singlet at δ_H 1.58 ($6H_3$) which corresponded to (H_3-25, H_3-26, H_3-27, H_3-28, H_3-29, H_3-30).

All proton signals have been further substantiated by the presence of the ^{13}C / DEPT experiments (Table 3, Figure 8), which showed 30 resonance carbons, due to eight methyls, ten methylenes, six methines resonating at δ_C 124.20, 124.30 and 124.40, and six trisubstituted quartemary carbons. In the ^{13}C-NMR spectrum, the out-of-chain methyl groups appeared at δ_C 17.65, 16.02 and 15.98 indicated the geometry of the six trisubstitutional double bonds, while signal appeared at δ_C 25.67 (H_3-1 and H_3-24), confirmed its in-chain position. The elucidation of the structure has been approved by 2D NMR COSY (Figure 9), HMQC (Figure 10), and GCMS (Figure 11). These NMR spectral data were almost the same as squalene.

Based on these ^{1}H and ^{13}C-NMR spectral features and by comparison with the authentic data, compound **DM2** was identified as squalene (Barrero et al., 1996; Miyazawa et al., 1996; Nishiyama et al., 1996).

Table 3: ^{1}H-NMR and ^{13}C-NMR, for squalene in $CDCl_3$

Position	δ_H (int.; mult.; J(Hz))	δ_C
1, 24	1.66 (3H, s)	25.67
2, 23		131.22
3, 22	5.10 (1H, m)	124.20
4, 21	2.05 (2H, m)	26.77
5, 20	2.05 (2H, m)	39.75
6, 19		134.88
7, 18	5.10 (1H, m)	124.40
8, 17	2.05 (2H, m)	26.66
9, 16	2.05 (2H, m)	
10, 15		135.08
11, 14	5.10 (1H, m)	124.30
12, 13	2.05 (2H, m)	28.27
25, 30	1.58 (3H, bs)	17.65
26, 29	1.58 (3H, bs)	16.02
27, 28	1.58 (3H, bs)	15.98

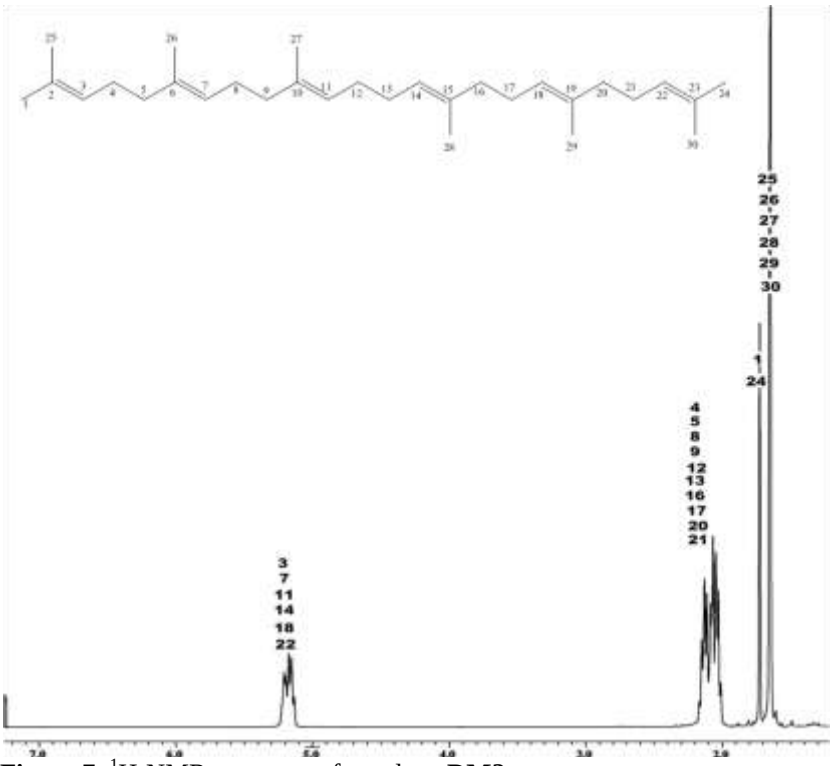
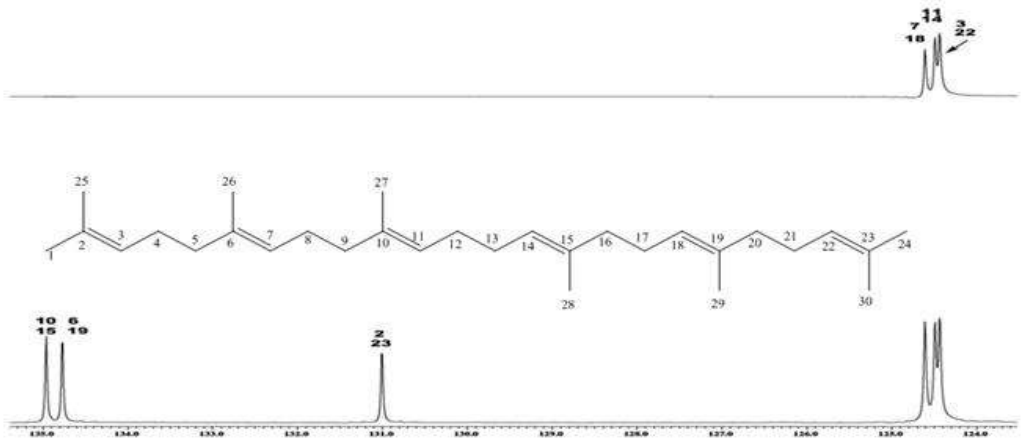
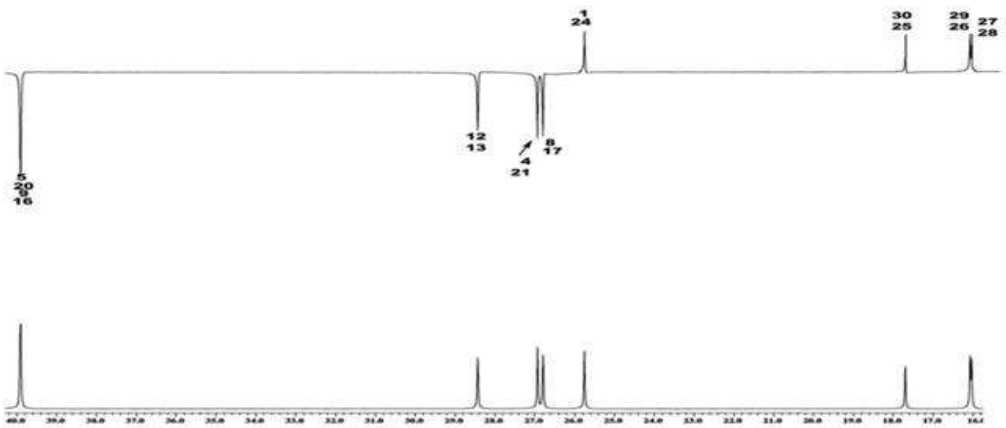


Figure 7: ^{1}H-NMR spectrum of squalene **DM2**



Expansion of the ^{13}C/DEPT spectrum in the sp^{2} region



Expansion of the ^{13}C/DEPT spectrum in the sp^{3} region

Figure 8: ^{13}C/DEPT spectra of squalene DM2

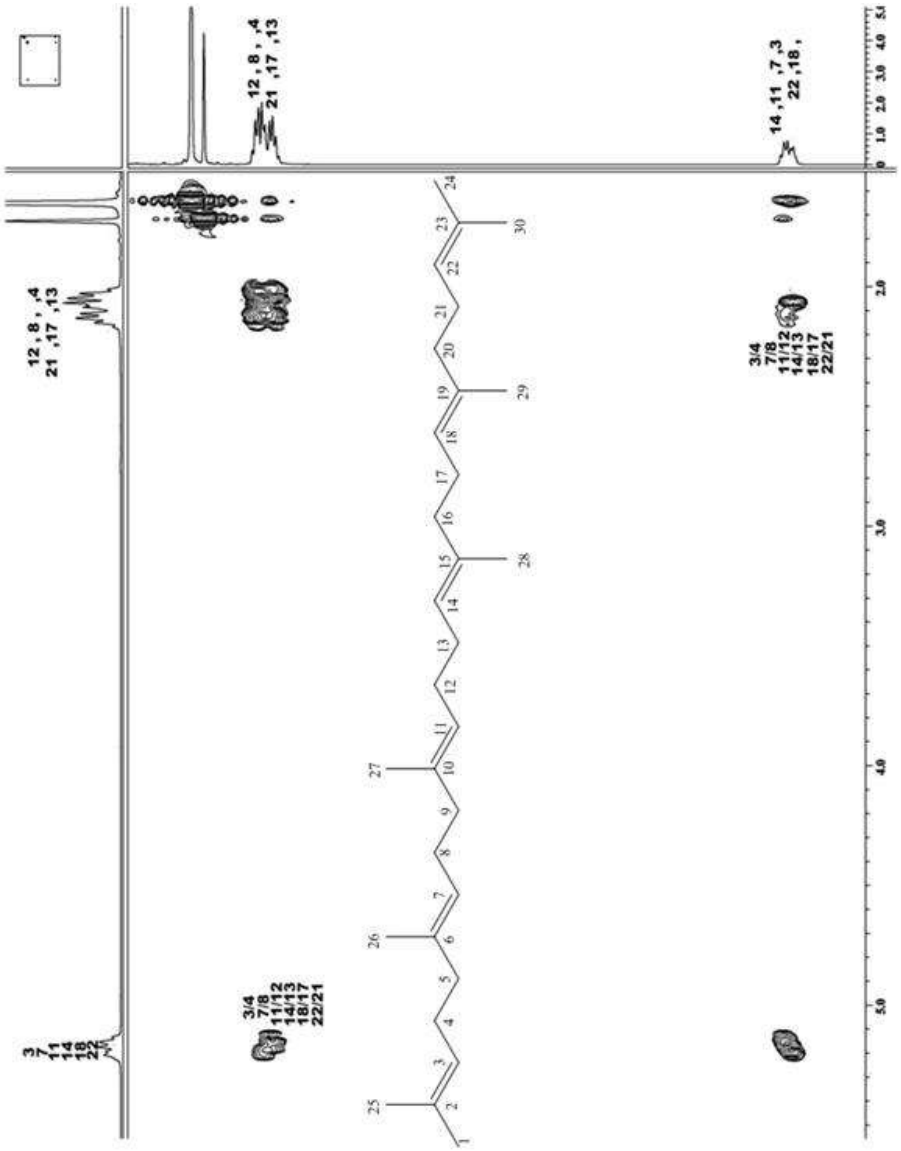


Figure 9: 1H-1H -COSY spectrum of squalene **DM2**

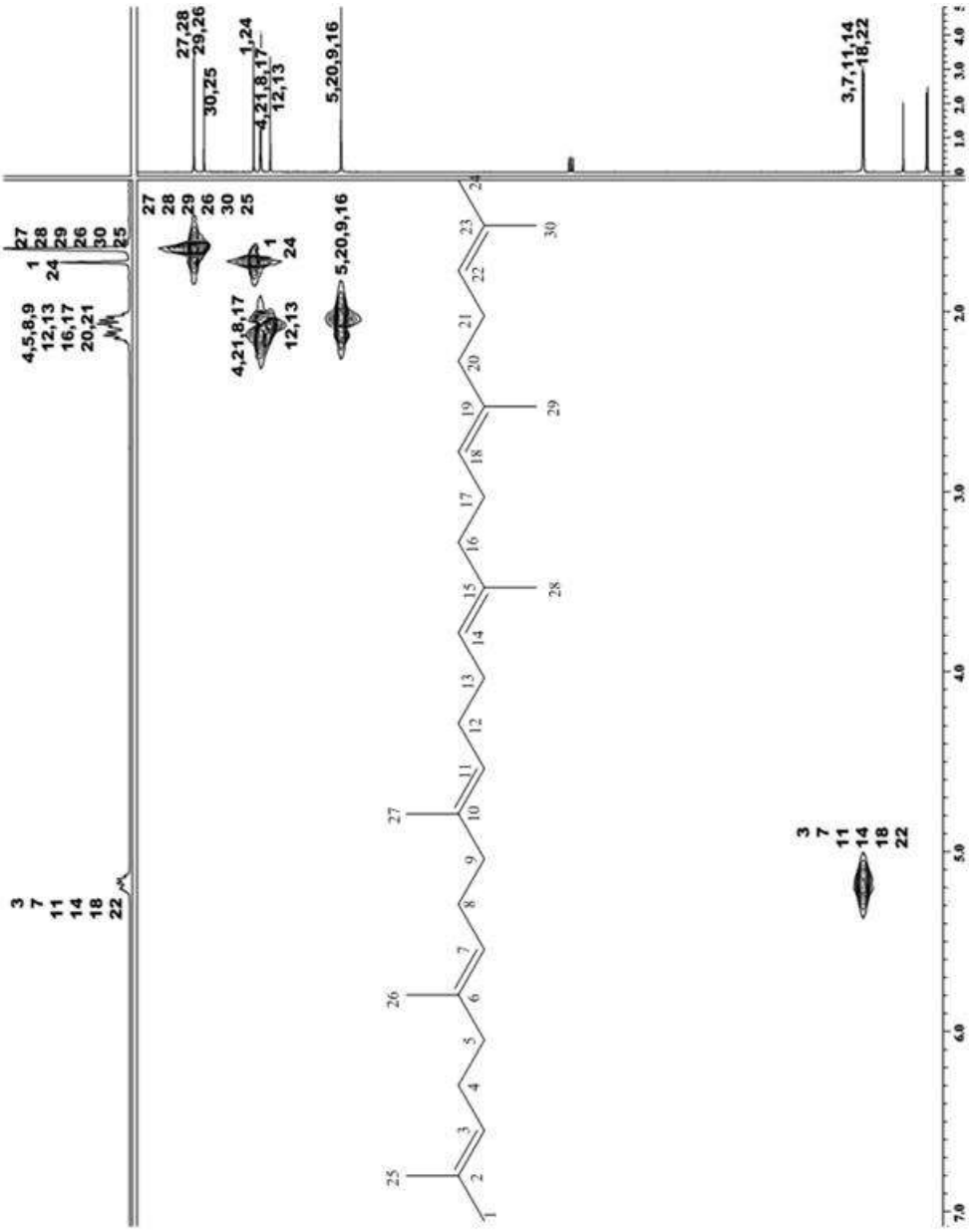


Figure 10: HMQC spectrum of squalene **DM2**

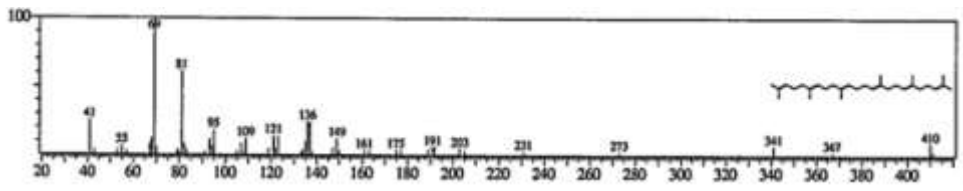
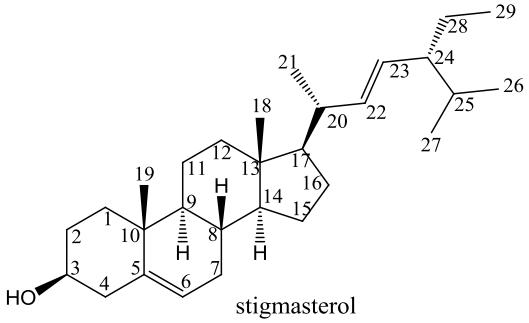
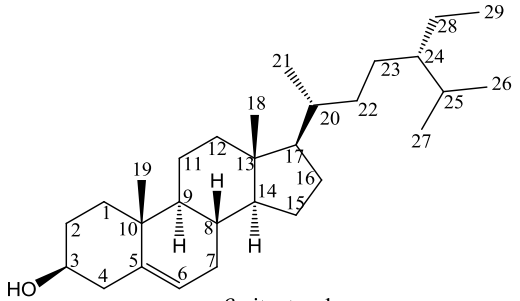


Figure 11: GCMS of squalene **DM2**

3.2.3 Stigmasterol DM3 and *β*-sitosterol DM4



stigmasterol



β-sitosterol

Compound **DM3** and **DM4** were isolated as a mixture of a white solid with the same R_f values. The UV spectrum showed absorption bands at λ_{max} 302 and 254 nm. The infrared (IR) spectrum indicated the presence of hydroxyl group by the absorption bands at ν_{max} 3430 cm^{-1}. The presence of stigmasterol **DM3** was confirmed by the LCMS (Figure 14) showing a molecular peak $[M+H]^+$ at *m/z* 413 and 415, corresponding to the molecular formula $C_{29}H_{49}O$ and $C_{29}H_{51}O$ respectively.

Sterols with an ethyl group at C-24, such as stigmasterol and *β*-sitosterol are by far the most abundant compounds in most plants.

The mixture of these two compounds was analyzed by ^1H and ^{13}C-NMR spectroscopy. The ^1H-NMR spectrum (Table 4, Figure 12) of this mixture showed twelve methyl groups (six methyl groups for each compound) resonated as singlets, doublets and triplets in the region of δ_H 0.65-1.00. In the ^1H-NMR spectrum, the signal of a methine proton attached to C-6 of both compounds resonated further downfield as a doublet at δ_H 5.32, the most significant differences on the ^1H-NMR chemical shift of these two molecules were the proton signals of C-22 and C-23. In the compound **DM3,** the presence of a double bond at position C-22 gave rise to two-doublet of a doublet signals at δ_H 4.96 and 5.09 which were belong to H-23, and H-22, respectively. In compound **DM4**, the protons of two ethylene groups C-22 and C-23, gave rise as multiplets in the region of δ_H 0.90- 2.00. The rest of the protons resonated as multiplets in the region of δ_H 0.7-3.5.

The integration of H-6, H-22 and H-23 appeared to be in the ratio of 1:0.25:0.25. Therefore, it could be deduced that the mixture of isolated stigmasterol and *β*-sitosterol was in the ratio of approximately 1:2.

Since compound **DM3** and **DM4** have an identical sterol skeleton, the ^{13}C/DEPT spectra (Table 4, Figure 13) of this mixture showed quite similar chemical shifts. The most significant differences on the chemical shift of these two molecules were the signals of C-22 and C-23. For compound **DM3**, the sp^2 carbons; C-22 and C-23 resonated at δ_C 138.3 and 129.2, respectively. The presence of the double bond also moved C-20, C-21, C-24, C-25 and C-28 further downfield at δ_C 40.5, 21.2, 51.2, 31.9 and 25.4 respectively, as compared to that of compound **DM4**, which showed the signals at δ_C 36.1, 18.8, 45.8, 29.1 and 23.1 for C-20, C-21, C-24, C-25 and C-28, respectively.

By comparing the NMR spectra data with the literature value (De-Eknamkul and Potduang, 2003; Forgo and Kövér, 2004; Huang and Kong, 2006; Lendl et al., 2005; Manoharan et al., 2005; Säynäjoki et al., 2003; Xu et al., 2005), it was confirmed that compound **DM3** was a stigmasterol and compound **DM4** was a *β*-sitosterol.

Table 4: 1D (^{1}H and ^{13}C) and 2D (HMBC) NMR Spectral Data of **DM3** and **DM4**

Position	δ_H (int.; mult.; J(Hz))	δ_C	δ_H (int.; mult.; J(Hz))	δ_C
1a	1.81 (1H, m)	37.2	1.81 (1H, m)	37.2
1b	1.04 (1H, m)		1.04 (1H, m)	
2a	1.79 (1H, m)	29.7	1.79 (1H, m)	31.6
2b	1.50 (1H, m)		1.50 (1H, m)	
3	3.51 (1H, m)	71.8	3.46 (1H, m)	71.8
4	2.27 (1H, m)	42.3	2.27 (1H, m)	39.8
5		140.7		140.7
6	5.32 (1H, m)	121.7	5.32 (1H, m)	121.7
7	1.93 (1H, m)	31.9	1.93 (1H, m)	31.9
	1.50 (1H, m)		1.50 (1H, m)	
8	1.45 (1H, m)	31.9	1.45 (1H, m)	31.9
9	0.92 (1H, m)	50.1	0.92 (1H, m)	50.1
10		36.5		36.5
11	1.50 (2H, m)	21.1	1.50 (2H, m)	21.1
12	1.95 (1H, m)	39.7	1.95 (1H, m)	42.3
	1.17(1H, m)		1.17 (1H, m)	
13		42.2		42.3
14	1.00 (1H, m)	56.8	1.00 (1H, m)	56.7
15	1.54 (1H, m)	24.3	1.54 (1H, m)	24.3
	1.04 (1H, m)		1.04 (1H, m)	
16	1.65 (1H, m)	28.9	1.65 (1H, m)	28.2
17	1.12 (1H, m)	55.9	1.12 (1H, m)	56.0
18	0.67 (3H, s)	12.0	0.65 (3H,s)	11.8
19	1.00 (3H, s)	19.4	1.00 (3H, s)	19.4
20	2.00 (1H, m)	40.5	1.95 (1H, m)	36.1
21	0.98 (3H, m)	21.2	0.98 (3H, m)	18.8
22	5.09 (1H, *d*, 15.1)	138.3	0.93 (1H, m)	33.9
			1.99 (1H, m)	
23	4.96 (1H, *d*, 15.1)	129.2	1.04 (1H, m)	26.0
24	1.52 (1H, m)	51.2	1.47 (1H, m)	45.8
25	1.53 (1H, m)	31.9	1.47 (1H, m)	29.1
26	0.83 (3H,m)	21.1	0.80 (3H, m)	19.8
27	0.80 (3H, m)	19.9	0.78 (3H, m)	19.0
28	1.43 (1H, m)	25.4	1.21 (1H, m)	23.0
	1.17 (1H, m)		1.11 (1H, m)	
29	0.81 (3H, t)	12.2	0.89 (3H, m)	12.0

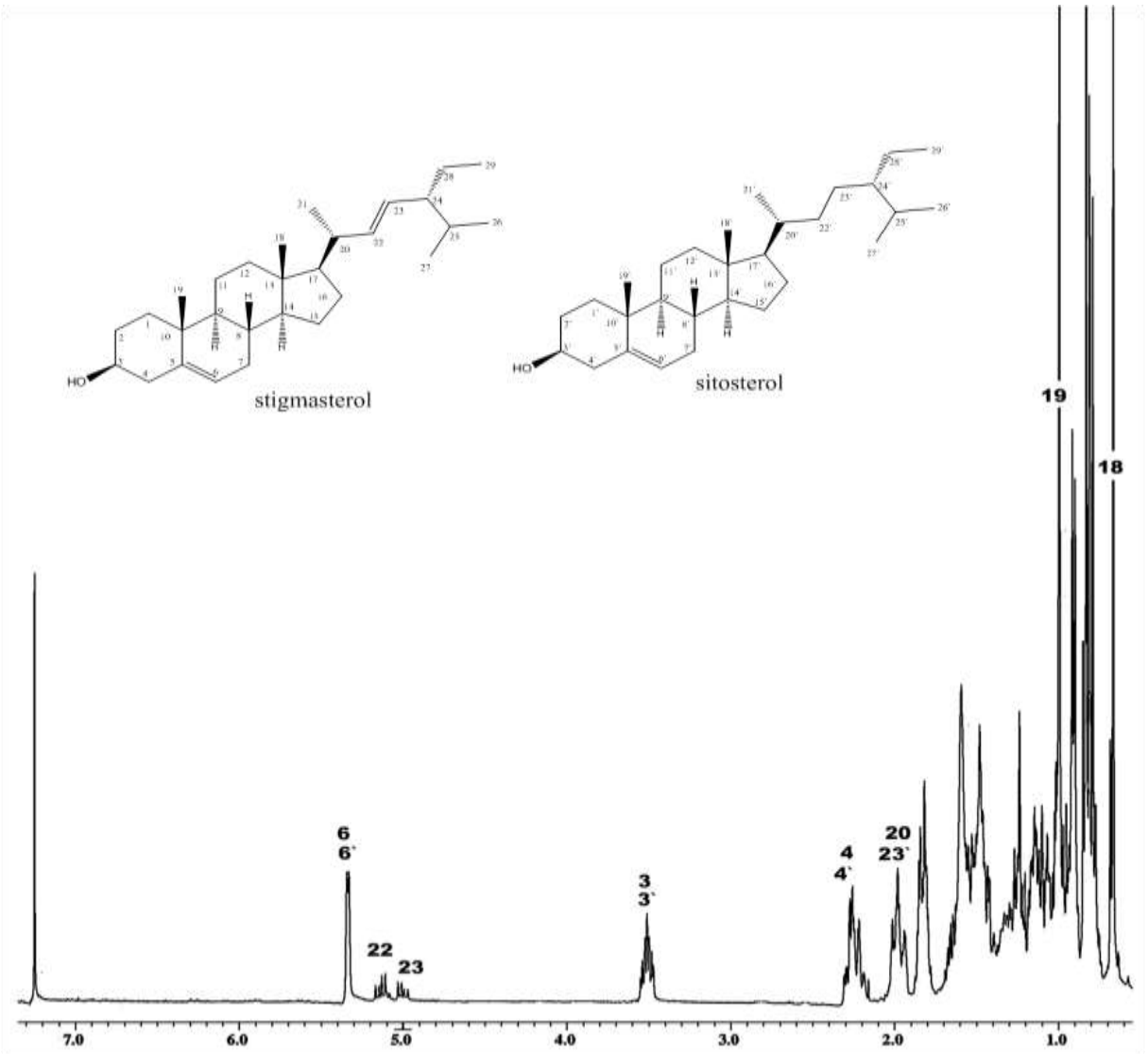


Figure 12: ^{1}H-NMR spectrum of a mixture of stigmasterol **DM3** and *β*-sitosterol **DM4**

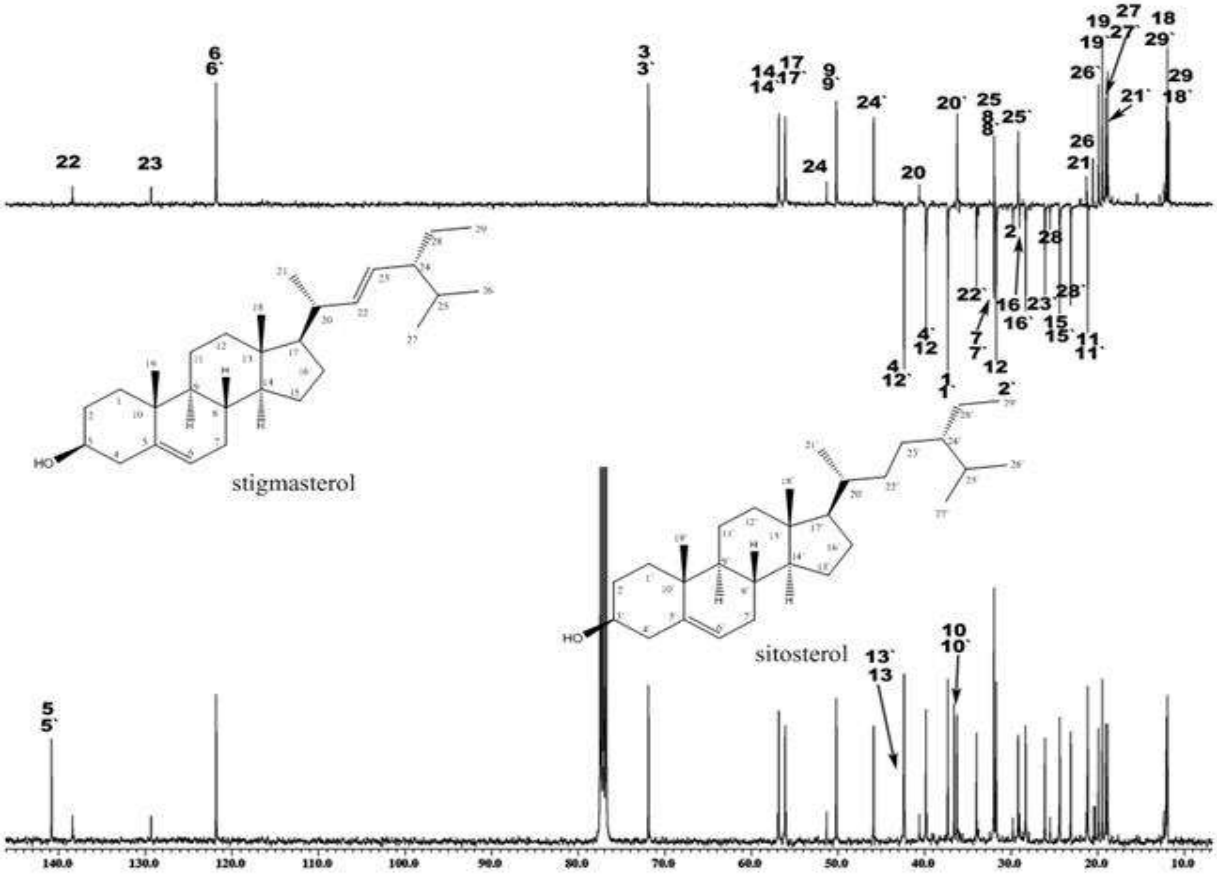


Figure 13: ^{13}C /DEPT NMR spectra of a mixture of stigmasterol **DM3** and β-sitosterol **DM4**

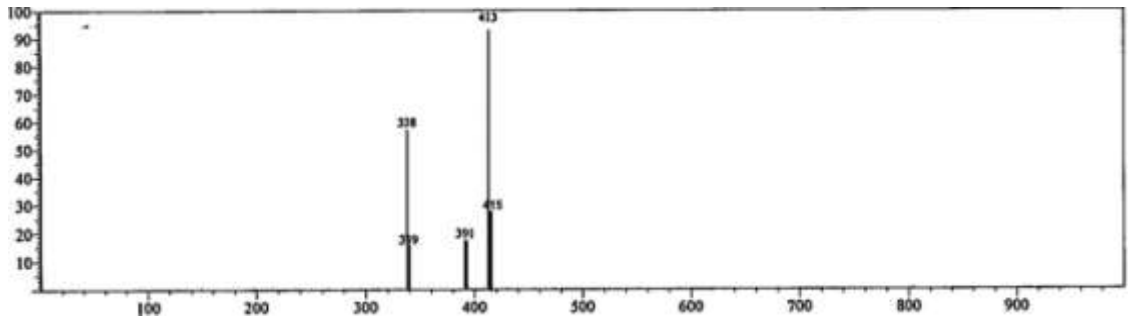


Figure 14: LCMS of stigmasterol **DM3** and *β*-sitosterol **DM4**

Experimental Part

General Methods

All solvents used in this experiment are distilled industrial grade. Silica gel 60, 230-400 mesh ASTM (Merck 9385) was used for column chromatography. A slurry of silica gel 60 (approximately 30:1 silica gel to sample ratio), PTLC silica gel 60 F_{254} glass plates of size 20 cm x 20 cm (Merck 1.05715.0001). The NMR spectra were obtained using JEOL LA400 FT NMR and JEOL ECA400 FT NMR Spectrometer System using deuterated chloroform as solvent. Chemical shifts were reported in ppm and coupling constants were given in Hertz (Hz). Mass spectra were carried out on Agilent Technologies 6530 Accurate-Mass Q-TOF LC/MS, with ZORBAX Eclipse XDB-C18 Rapid Resolution HT 4.6 mm i.d. x 50 mm x 1.8 µm column, the EI MS spectra were obtained on Shimadzu GC-MS QP2000A spectrometer 70 eV , the high-resolution ESI MS were measured on a LTQ Orbitrap XL (Thermo Scientific). UV spectra were recorded on a Shimadzu UV-Visible Recording Spectrophotometer using HPLC grade ethanol as solvent with mirror UV cell. The infrared (IR) spectra were obtained through Perkin Elmer FT-IR Spectrometer Spectrum RX1 using chloroform as solvent. Melting points were taken

on hot stage Gallen Kamp melting point apparatus and were uncorrected; all the above experiments have been done in Department of Chemistry, Faculty of Science, University of Malaya (UM), Kuala Lumpur, Malaysia.

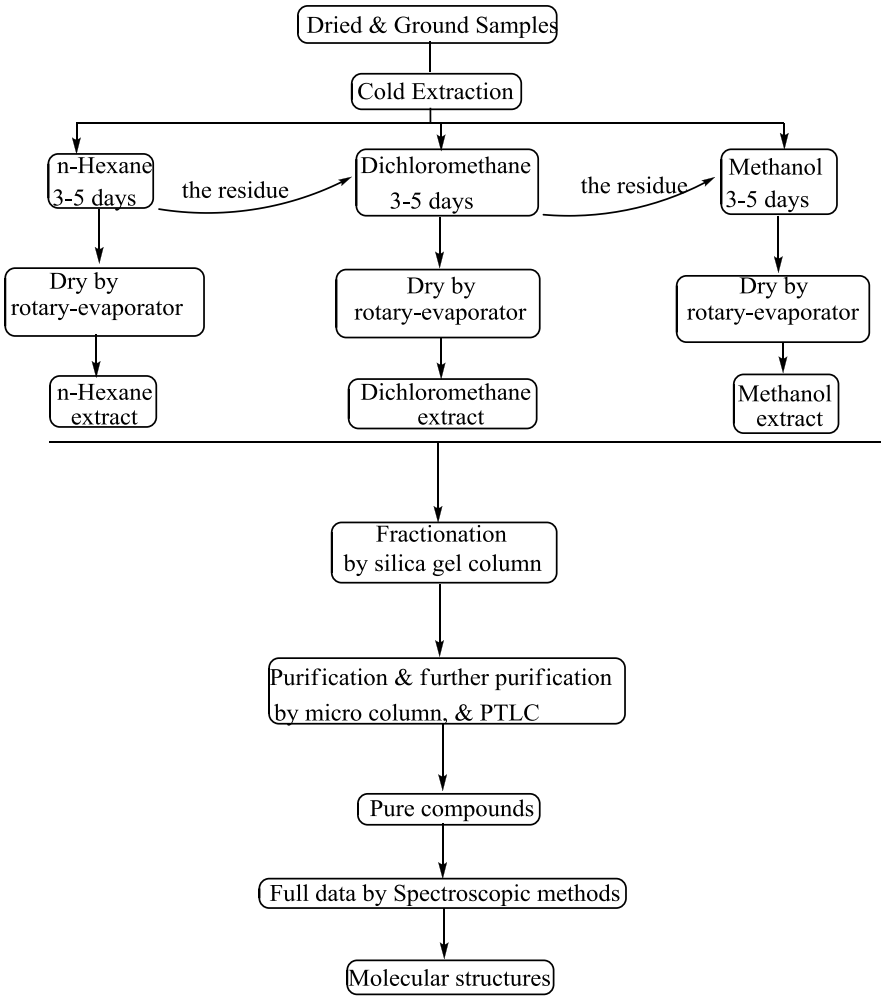
Extraction and Isolation of *Dysoxylum Macrocarpum*

The dried samples of *Dysoxylum macrocarpum* were ground and the extraction was carried out by cold percolation method using *n*-hexane, dichloromethane (CH_2Cl_2) and methanol (MeOH) (scheme 1). Initially, the dried samples were extracted by using *n*-hexane for 3 to 5 days. The *n*-hexane eluent was dried using the rotary-evaporator. The *n*-hexane solvent was replaced by CH_2Cl_2 and MeOH for another 3-5 days respectively by applying the same methods as above.

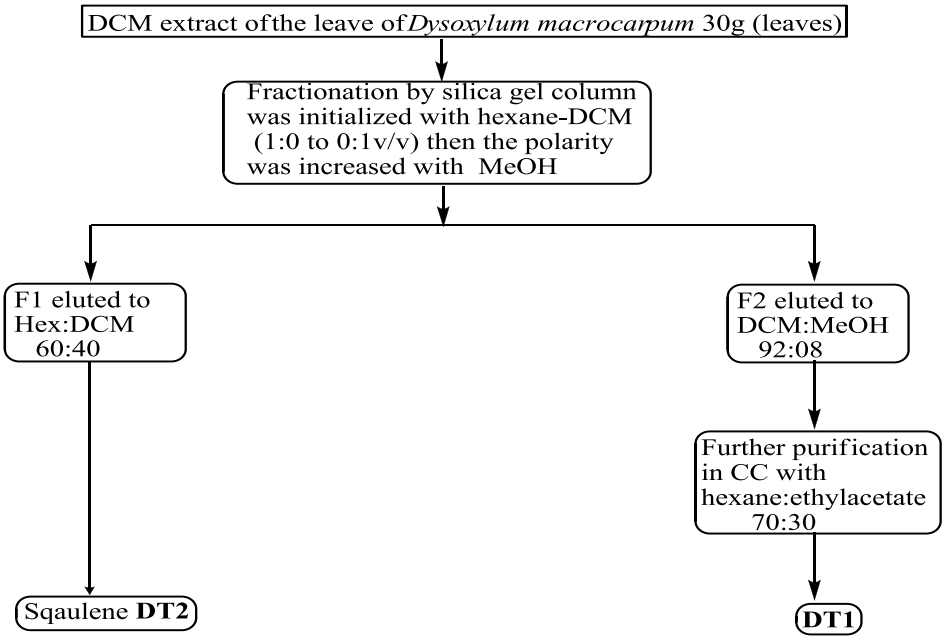
The dichloromethane crudes were subjected to column chromatography and preparative thin layer chromatography to yield four compounds. Two compounds were isolated from the leave of *Dysoxylum macrocarpum*, which are 5-Hydroxy-7-methoxy-2-methyl-4H-chromen-4-one (Eugenin) **DM1**. This compound was new as crystal and squalene **DM2**. Two more compounds were isolated from the bark of *Dysoxylum macrocarpum*; they were stigmasterol **DM3** and sitosterol **DM4**, as shown in Table 5 and in schemes 2, 3.

Table 5 : Chemical constituents of *Dysoxylum macrocarpum*

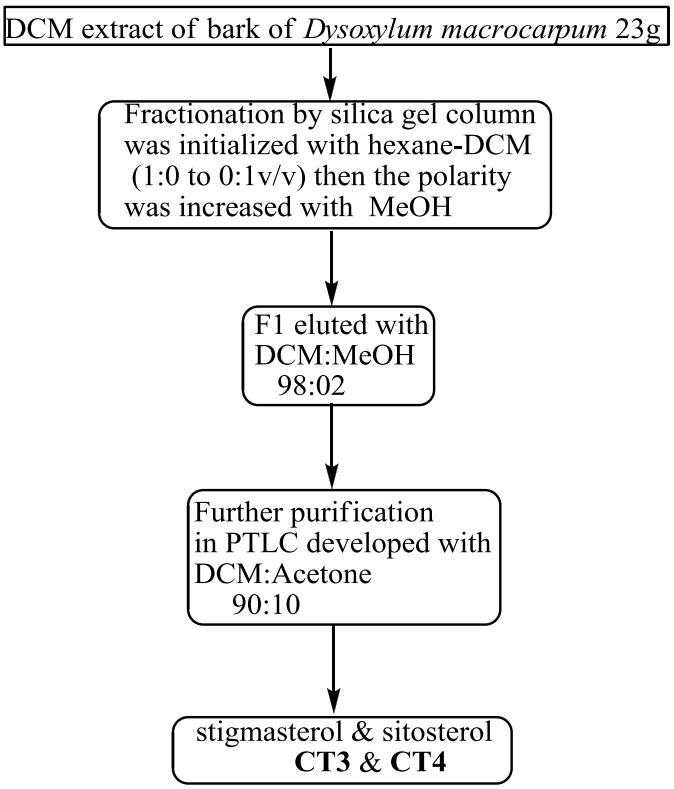
Compounds	Type of compounds	%yield
(Eugenin) **DM1**	phenolic	0.18
Squalene **DM2**	triterpene	1.2
Stigmasterol **DM3** and sitosterol **DM4**	steroids	0.23



Scheme 1: General **e**xtraction of chemical constituents from *Dysoxylum macrocarpum*



Scheme 2: Isolation and purification of Eugenin **DT1** and Squalene **DT2** from the leave *Dysoxylum macrocarpum*



Scheme 3: Isolation and purification of stigmasterol and sitosterol from the bark of *Dysoxylum macrocarpum*

Plant Materials

The plant materials were collected and identified from Jeli – Dabong, Kelantan, Malaysia in 1993 by the team of Herbarium of Chemistry Department, University of Malaya, Kuala Lumpur, Malaysia. It has been deposited in the above herbarium under voucher specimens KL 4302.

References

Aalbersberg W., Singh Y. (1991) Dammarane triterpenoids from Dysoxylum richii. Phytochemistry 30:921-6.

Barrero A.F., Oltra J.E., Poyatos J.A. (1996) Acidic metabolites from Phycomyces blakesleeanus. Phytochemistry 42:1427-1433.

Bhat S.V., Shah V., Dohadwalla A.N., Mandrekar S.S., De S.N.J. (1986) Isolation of a pharmacologically active substance having the structure of a chromone alkaloid from plant(s) belonging to the Meliaceae family, Hoechst India Ltd. pp. 21 pp.

Cui B.-S., Ma X.-Q., Yang D.-H., Hu X.-Q., Cai S.-Q. (2008) Purification method of rohitukine from the stem bark of Dysoxylum binectariferum. J. Chin. Pharm. Sci. 17:153-157.

De-Eknamkul W., Potduang B. (2003) Biosynthesis of [beta]-sitosterol and stigmasterol in Croton sublyratus proceeds via a mixed origin of isoprene units. Phytochemistry 62:389-398.

Duh C.-Y., Wang S.-K., Chen I.-S. (2000a) Cytotoxic Prenyleudesmane Diterpenes from the Fruits of Dysoxylum kuskusense. J. Nat. Prod. 63:1546-1547. DOI: 10.1021/np000264z.

Duh C.Y., Wang S.K., Chen I.S. (2000b) Cytotoxic prenyleudesmane diterpenes from the fruits of Dysoxylum kuskusense. J Nat Prod 63:1546-7.

Forgo P., Kövér K.E. (2004) Gradient enhanced selective experiments in the 1H NMR chemical shift assignment of the skeleton and side-chain resonances of stigmasterol, a phytosterol derivative. Steroids 69:43-50.

Fujioka T., Yamamoto M., Kashiwada Y., Fujii H., Mihashi K., Ikeshiro Y., Chen I.-S., Leed K.-H. (1998a) Antitumor agents. 182. Novel cytotoxic diterpenes from the stem of Dysoxylum kuskusense. Bioorg. Med. Chem. Lett. 8:3479-3482.

Fujioka T., Yamamoto M., Kashiwada Y., Fujii H., Mihashi K., Ikeshiro Y., Chen I.S., Lee K.H. (1998b) Novel cytotoxic diterpenes from the stem of Dysoxylum kuskusense. Bioorg Med Chem Lett 8:3479-82.

Govindachari T.R., Suresh G., Kumari G.N.K. (1994) Triterpenoids from Dysoxylum malabaricum. Phytochemistry 37:1127-9.

Govindachari T.R., Kumari G.N.K., Suresh G. (1996) Ergosta-5,24(24')-diene-3β,4β,20S-triol, an ergostane steroid from Dysoxylum malabaricum. Phytochemistry 44:153-155.

Govindachari T.R., Suresh G., Kumari G.N.K., Rajamannar T., Partho P.D. (1999) Nymania-3: a bioactive triterpenoid from Dysoxylum malabaricum. Fitoterapia 70:83-86.

He K., Timmermann B.N., Aladesanmi A.J., Zeng L. (1996) A biflavonoid from Dysoxylum lenticellare gillespie. Phytochemistry 42:1199-1201.

He X.-F., Wang X.-N., Yin S., Dong L., Yue J.-M. (2009) Ring A Modified Novel Triterpenoids from Dysoxylum hainanense. Eur. J. Org. Chem.:4818-4824. DOI: 10.1002/ejoc.200900609.

He X.-F., Wang X.-N., Gan L.-S., Yin S., Dong L., Yue J.-M. (2008a) Two Novel Triterpenoids from Dysoxylum hainanense. Org. Lett. 10:4327-4330. DOI: 10.1021/ol801834y.

He X.-F., Wang X.-N., Gan L.-S., Yin S., Dong L., Yue J.-M. (2008b) Two novel triterpenoids from Dysoxylum hainanense. Org Lett 10:4327-30.

Hisham A., Bai M.D.A., Fumimoto Y., Hara N., Shimada H. (1996) Complete 1H and 13C NMR spectra assignment of cabraleadiol, a dammarane triterpene from Dysoxylum malabaricum Bedd. Magn. Reson. Chem. 34:146-50.

Hisham A., Ajitha B.M.D., JayaKumar G., Nair M.S., Fujimoto Y. (2001) Triterpenoids from Dysoxylum malabaricum. Phytochemistry 56:331-334.

Huang R., Harrison L.J., Sim K.-Y. (1999) A triterpenoid with a novel abeo-dammarane skeleton from Dysoxylum cauliflorum. Tetrahedron Lett. 40:1607-1610.

Huang X., Kong L. (2006) Steroidal saponins from roots of Asparagus officinalis. Steroids 71:171-176.

Ismail I.S., Nagakura Y., Hirasawa Y., Hosoya T., Lazim M.I.M., Lajis N.H., Morita H. (2009) Acutaxylines A and B, two novel triterpenes

from Dysoxylum acutangulum. Tetrahedron Lett. 50:4830-4832. DOI: 10.1016/j.tetlet.2009.05.110.

Jogia M.K., Andersen R.J. (1987) Dysoxylin, a limonoid from Dysoxylum richii. Phytochemistry 26:3309-11.

Jogia M.K., Andersen R.J. (1989) Limonoids from the Fijian medicinal plant Dysoxylum richii. Can. J. Chem. 67:257-60.

Jogia M.K., Andersen R.J., Mantus E.K., Clardy J. (1989) Dysoxysulfone, a sulfur rich metabolite from the Fijian medicinal plant Dysoxylum richii. Tetrahedron Lett. 30:4919-20.

Lakdawala A.D., Shirole M.V., Mandrekar S.S., Dohadwalla A.N. (1988) Immunopharmacological potential of rohitukine: a novel compound isolated from the plant Dysoxylum binectariferum. Asia Pac. J. Pharmacol. 3:91-8.

Lendl A., Werner I., Glasl S., Kletter C., Mucaji P., Presser A., Reznicek G., Jurenitsch J., Taylor D.W. (2005) Phenolic and terpenoid compounds from Chione venosa (sw.) urban var. venosa (Bois Bandé). Phytochemistry 66:2381-2387.

Luo X.-D., Wu S.-H., Wu D.-G., Ma Y.-B., Qi S.-H. (2002a) Novel antifeeding limonoids from Dysoxylum hainanense. Tetrahedron 58:7797-7804.

Luo X.-D., Wu S.-H., Wu D.-G., Ma Y.-B., Qi S.-H. (2002b) Three new apo-tirucallols with six-membered hemiacetal from Meliaceae. Tetrahedron 58:6691-6695.

Luo X.-D., Wu S.-H., Yang X.-S., Teng R.-W., Ma Y.-B., Wu D.-G., Hao X.-J., Lu Y., Liu X.-Y., Zheng Q.-T. (2000a) Prieurianin-type tetranortriterpenoids from the bark of Dysoxylum hainanense. Heterocycles 53:2225-2232.

Luo X.D., Wu S.H., Ma Y.B., Wu D.G. (2000b) Tirucallane triterpenoids from Dysoxylum hainanense. Phytochemistry 54:801-805.

Luo X.D., Wu S.H., Ma Y.B., Wu D.G. (2000c) Tirucallane triterpenoids from Dysoxylum hainanense. Phytochemistry 54:801-5.

Luo X.D., Wu S.H., Ma Y.B., Wu D.G. (2001a) Ent-pimarane derivatives from Dysoxylum hainanense. Phytochemistry 57:131-134.

Luo X.D., Wu S.H., Ma Y.B., Wu D.G. (2001b) ent-Pimarane derivatives from Dysoxylum hainanense. Phytochemistry 57:131-4.

Mabberly D.J., Pannell C.M. (1989) Meliaceae in Tree Flora of Malaya, Kuala Lumpur

Manoharan K.P., Benny T.K.H., Yang D. (2005) Cycloartane type triterpenoids from the rhizomes of Polygonum bistorta. Phytochemistry 66:2304-2308.

Miyazawa M., Nankai H., Kameoka H. (1996) Biotransformation of acyclic terpenoid (2E,6E)-farnesol by plant pathogenic fungus Glomerella cingulata. Phytochemistry 43:105-109.

Mohamad K., Martin M.-T., Litaudon M., Gaspard C., Sevenet T., Pais M. (1999) Tirucallane triterpenes from Dysoxylum macranthum. Phytochemistry 52:1461-1468.

Mohanakumara P., Sreejayan N., Priti V., Ramesha B.T., Ravikanth G., Ganeshaiah K.N., Vasudeva R., Mohan J., Santhoshkumar T.R., Mishra P.D., Ram V., Shaanker R.U. (2010) Dysoxylum binectariferum Hook.f (Meliaceae), a rich source of rohitukine. Fitoterapia 81:145-148. DOI: 10.1016/j.fitote.2009.08.010.

Mulholland D.A., Naidoo N. (2000) Dammarane triterpenoids from Dysoxylum muellerii. Biochem. Syst. Ecol. 28:295-297.

Mulholland D.A., Nair J.J., Taylor D.A.H. (1996) Glabretal triterpenoids from Dysoxylum muelleri. Phytochemistry 42:1667-1671.

Mulholland D.A., Iourine S., Taylor D.A.H. (1998) Sesquiterpenoids from Dysoxylum schiffneri. Phytochemistry 47:1421-1422.

Mulholland D.A., Monkhe T.V., Pegel K.H., Taylor D.A.H. (1999) Limonoids and diterpenoids from Dysoxylum spectabile (Meliaceae). Biochem. Syst. Ecol. 27:313-315.

Naik R.G., Kattige S.L., Bhat S.V., Alreja B., De S.N.J., Rupp R.H. (1988) An antiinflammatory and immunomodulatory piperidinylbenzopyranone from Dysoxylum binectariferum: isolation, structure and total synthesis. Tetrahedron 44:2081-6.

Najmuldeen I.A., Hadi A.H.A., Awang K., Mohamad

K., Ng S.W. (2008) 17-(5-Ethyl-6-methylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthrene-3,7-diol from Chisocheton tomentosus (Meliaceae). Acta Crystallogr., Sect. E: Struct. Rep. Online E64:o2163, o2163/1-o2163/16. DOI: 10.1107/s1600536808033163.

Najmuldeen I.A., Abdul H.A.H., Awang K., Mohamad K., Ng S.W. (2010a) 5-Hydroxy-7-methoxy-2-methyl-4H-chromen-4-one from Dysoxylum macrocarpum (Meliaceae). Acta Crystallogr., Sect. E: Struct. Rep. Online E66:o1883. DOI: 10.1107/s1600536810025146.

Najmuldeen I.A., Abdul H.A.H., Awang K., Mohamad K., Ng S.W. (2010b) 5-Hydroxy-7-methoxy-2-methyl-4H-chromen-4-one from Dysoxylum macrocarpum (Meliaceae). Acta Crystallogr., Sect. E: Struct. Rep. Online E66:o1883. DOI: 10.1107/s1600536810025146.

Najmuldeen I.A., Abdul H.A.H., Awang K., Mohamad K., Ng S.W. (2010c) 14-Deoxyxyloccensin K from Chisocheton ceramicus (Meliaceae). Acta Crystallogr., Sect. E: Struct. Rep. Online E66:o1927. DOI: 10.1107/s160053681002564x.

Najmuldeen I.A., Hadi A.H.A., Awang K., Mohamad K., Ketuly K.A., Mukhtar M.R., Chong S.-L., Chan G., Nafiah M.A., Weng N.S., Shirota O., Hosoya T., Nugroho A.E., Morita H. (2011) Chisomicines A-C, Limonoids from Chisocheton ceramicus. J. Nat. Prod. 74:1313-1317. DOI: 10.1021/np200013g.

Nishiyama Y., Moriyasu M., Ichimaru M., Tachibana Y., Kato A., Mathenge S.G., Nganga J.N., Juma F.D. (1996) Acyclic triterpenoids from Ekebergia capensis. Phytochemistry 42:803-807.

Russell G.B., Hunt M.B., Bowers W.S., Blunt J.W. (1994) A sesquiterpenoid ant repellent from Dysoxylum spectabile. Phytochemistry 35:1449-53.

Säynäjoki S., Sundberg S., Soupas L., Lampi A.-M., Piironen V. (2003) Determination of stigmasterol primary oxidation products by high-performance liquid chromatography. Food Chemistry 80:415-421.

Singh Y., Aalbersberg W. (1992) Dammarane triterpenoids from a Fijian medicinal plant, Dysoxylum richii. Part 2. Dammarane triterpenoids from Dysoxylum richii. Phytochemistry 31:4033-5.

Tsui W.-Y., Brown G.D. (1996) Chromones and chromanones from Baeckea frutescens. Phytochemistry 43:871-876. DOI: 10.1016/0031-9422(96)00360-3.

Xie B.-J., Yang S.-P., Yue J.-M. (2008a) Terpenoids from Dysoxylum densiflorum. Phytochemistry (Elsevier) 69:2993-2997. DOI: 10.1016/j.phytochem.2008.09.017.

Xie B.-J., Yang S.-P., Yue J.-M. (2008b) Terpenoids from Dysoxylum densiflorum. Phytochemistry 69:2993-7.

Xu W.L., Huang Y.B., Qian J.H., Sha O., Wang Y.Q. (2005) Separation and purification of stigmasterol and [beta]-sitosterol from phytosterol mixtures by solvent crystallization method. Separation and Purification Technology 41:173-178.

Yang D.-H., Cai S.-Q., Zhao Y.-Y., Liang H. (2004) A new alkaloid from Dysoxylum binectariferum. J. Asian Nat. Prod. Res. 6:233-236. DOI: 10.1080/10286020310001608930.

الملخص

تم استخلاص اربع مركبات عضوية من قشور وأوراق شجرة ال *Dysoxylum macrocarpum* (Meliaceace) منها (5-hydroxy-7-methoxy-2-methyl-4*H*-chromen-4-one (Eugenin) **DM1**) و **DM2** squalene من أوراق الشجر ويعتبر ال **DM1** (Eugenin) مركبا جديدا من ناحية الشكل البلوري. بينما تم استخلاص **DM3** stigmasterol و ال **DM4** β-sitosterol من قشور الشجر.

و تضمن البحث الحالي عمليات استخلاص و فصل و تنقية المركبات مستخدمين عمود الفصل CC و من ثم PTLC. و تم تشخيص المركبات بعدة طرق طيفية منها UV و IR و MS (HRMS و GCMS و LCMS) و 1D-NMR(^{1}H-NMR و ^{13}C-NMR و DEPT) و 2D-NMR منها (COSY و HMQC و HMBC) و single crystal X-ray diffraction analysis

پوختە

چار ئاوێتێن ئەندامی هاتنە جودا کرن ژ تیڤلك و بەلکێن دارا *Dysoxylum macrocarpum* (Meliaceace) **ژ وان** (5-hydroxy-7-methoxy-2-methyl-4*H*-chromen-4-one (Eugenin) DM1) **و** squalene DM2 **ژ بەلکێن دارا, دیار بوو کو** (Eugenin) DM1 **ئاوێتەکێ نییە ژ لایێ شێوێ کریستالیڤە .**

بەلێ stigmasterol DM3 **و** β-sitosterol DM4 **هاتنە جودا کرن ژ تیڤلکێن دارا.**

و CC**ئەوژی ب هاریکاریا ل ڤەکۆلینا نوکە کریارێت پوختەکرن و ژێك جوداکرن و پاقژ کرن بخوڤە دگریت و** IR **و** UV. **وئەڤان ئاوێتان هاتنە دەست نیشانکرن ب گەلەك رێکێن شەبەنگی ژ وان** PTLC **و** DEPT **و** 1D-NMR**و** (^{1}H- NMR) **و** LCMS **و** GCMS **و** HRMS **(ژ وان** MS **و** ^{13}CNMR **(** 2D-NMR **ژ وان**) COSY **و** HMQC **و** HMBC **(** single crystal X-ray diffraction analysis.