SOME COMPOUNDS FROM FLOWER OF *WEDELIA TRILOBATA* (L.) HITCH. (ASTERACEAE)

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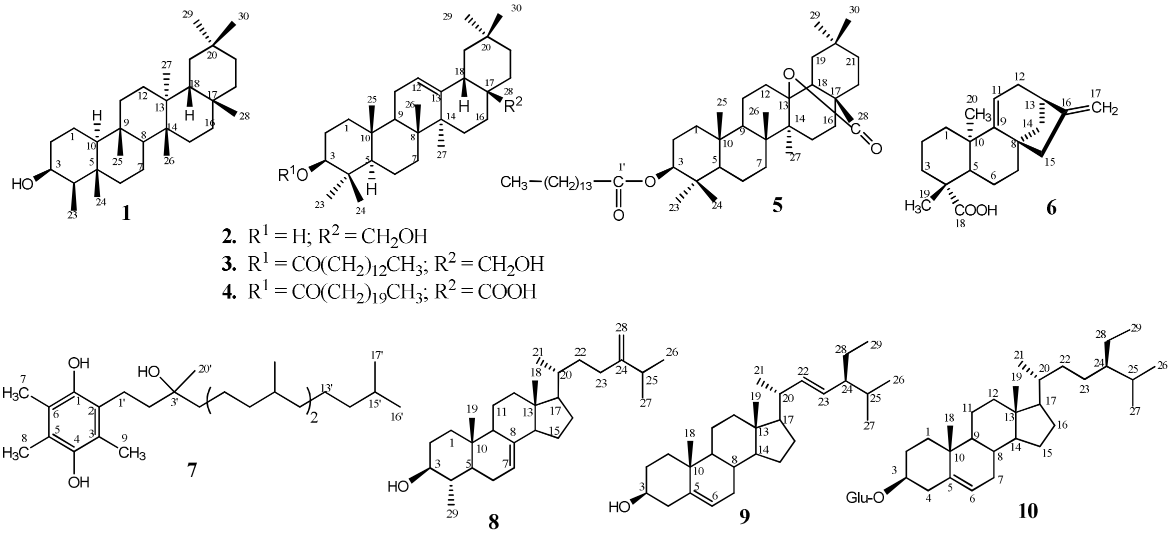
ABSTRACT: *Wedelia trilobata Hitch. has not much been chemically and biologically studied. From the flower of Wedelia trilobata, ten compounds were isolated: friedelan-3β-ol (or epifriedelanol) (1), erythrodiol (2), 28-hydroxy-3β -tetradecanoyloxyolean-12-ene (or 3β -tetradecanoyloxyerythrodiol) (3), 3β-henicosanoyloxyolean-12-en-28-oic acid (4), 3β-pentadecanoyloxyolean-28(13)-lactone (5), kaura-9(11),16-dien-18-oic acid (or grandiflorenic acid) (6), 2-(3-hydroxy-3,7,11,15-tetramethylhexadecyl)-3,5,6-trimethyl-1,4-dihydroxybenzene (or tocopherolhydroquinone) (7), gramisterol (8), stigmasterol (9) and β-sitosterol 3-O-β-D-glucopyranoside (10). The structures of these compounds were determined through the interpretation of their NMR data and compared with references.*

Key words: *Asteraceae, Wedelia trilobata, triterpenoids, diterpenoids, sterols.*



1

2. $R^1 = H$; $R^2 = CH_2OH$
3. $R^1 = CO(CH_2)_{12}CH_3$; $R^2 = CH_2OH$
4. $R^1 = CO(CH_2)_{19}CH_3$; $R^2 = COOH$

5

6

7

8

9

10

INTRODUCTION

Wedelia trilobata Hitch. is a plant of Asteraceae family that wildly grows in many areas in Vietnam. This herb was used as folk remedy for haemostatic, treating diarrhea, snake bite, wound, ect.[1] Moreover, its flowers have not much been chemically and biologically studied, therefore they were chosen to study. In this paper, we report the isolation and structural determination of ten compounds: friedelan-3β-ol (or epifriedelanol) **(1)**, erythrodiol **(2),** 28-hydroxy-3β-tetradecanoyloxyolean-12-ene (or 3β-tetradecanoyloxyerythrodiol) **(3)**, 3β-henicosanoyloxyolean-12-en-28-oic acid **(4)**, 3β-pentadecanoyloxyolean-28(13)-lactone **(5),** kaura-9(11),16-dien-18-oic acid (or grandiflorenic acid) **(6),** 2-(3-hydroxy-3,7,11,15-tetramethylhexadecyl)-3,5,6-trimethyl-1,4-dihydroxybenzene (or tocopherolhydroquinone) **(7)**, gramisterol **(8),** stigmasterol **(9)** and β-sitosterol 3-*O*-β-D-glucopyranoside **(10)**.

EXPERIMENTAL

General

The NMR spectra were measured on a Bruker Avance spectrometer, at 500 MHz for ^{1}H and 125 MHz for ^{13}C, in the Institute of Chemistry, Vietnam Academy of Science and Technology. The HR-ESI-MS were recorded on a HR-ESI-MS MicroOTOF–Q mass spectrometer in the University of Science, National University – HCM City.

Plant material

Flowers of plant were collected in Ho Chi Minh city, Vietnam in November 2008. The scientific name of plant was identified by pharmacist Phan Duc Binh, Associate Editor-in-Chief of the Journal of Drug and Health. A voucher specimen (No US-B002) was deposited in the herbarium of the Department of Organic Chemistry, University of Science, National University - Ho Chi Minh City.

Extraction and isolation

Fresh flower (10.3 kg) was washed, dried, ground into powder (950g) and extracted by maceration with ethanol at room temperature then the extracted solution was evaporated in reduced pressure to give methanol residue (302.6g). This crude extract was suspended in water and partitioned against petroleum ether to afford petroleum ether residue (**E**, 70 g) and water residue. The **E** residue was subjected to silica gel column chromatography, eluted with gradient solvent system of petroleum ether: chloroform (10: 0 to 0: 10) to obtain **1**, **3**, **4**, **5**, **7**, **8** and **9,** then chloroform:methanol (10: 0 to 0:10) to afford **2**, **6** and **10**.

Friedelan-3β-ol (or epifriedelanol) (1). White crystal, mp. 268-270^{o}C ($CHCl_3$). The ^{1}H-NMR, $CDCl_3$, δppm: 3.73 (1H, *s*, H-3), 0.93 (3H, *s*, H-23), 0.96 (3H, *s*, H-24), 0.86 (3H, *s*, H-25), 0.99 (3H, *s*, H-26), 1.00 (3H, *s*, H-27), 1.17 (3H, *s*, H-28), 0.99 (3H, *s*, H-29), 0.94 (3H, *s*, H-30). The ^{13}C-NMR, $CDCl_3$, δppm (Table 1).

Erythrodiol (2). White powder, mp. 164-166^{o}C ($CHCl_3$). The ^{1}H-NMR, $CDCl_3$, δppm: 5.19 (1H, *t*, 4.0, H-12), 3.22 (1H, *d*, 11.0, H-3), 3.22 (1H, *d*, 11.0, H-28a), 3.55 (1H, *d*, 11.0, H-28b), 0.99 (3H, *s*, H-23), 0.79 (3H, *s*, H-24), 0.93 (3H, *s*, H-25), 0.94 (3H, *s*, H-26), 1.16 (3H, *s*, H-27), 0.88 (3H, *s*, H-29), 0.87 (3H, *s*, H-30). The ^{13}C-NMR, $CDCl_3$, δppm (Table 1).

28-Hydroxy-3β-tetradecanoyloxyolean-12-ene (3). White powder, mp. 127-128^{o}C ($CHCl_3$). The ^{1}H-NMR, $CDCl_3$, δppm: 5.19 (1H, *t*, 3.5, H-12), 4.50 (1H, *m*, H-3), 3.21 (1H, *d*, 11.0, H-

28a), 3.54 (1H, *d*, 11.0, H-28b), 0.86 (3H, *s*, H-23), 0.86 (3H, *s*, H-24), 0.96 (3H, *s*, H-25), 0.94 (3H, *s*, H-26), 1.16 (3H, *s*, H-27), 0.89 (3H, *s*, H-29), 0.87 (3H, *s*, H-30), 2.29 (2H, *t*, 7.0, H-2’), 0.88 (3H, H-14’). The ^{13}C-NMR, CDCl$_3$, δppm (table 1).

3β-Henicosanoyloxyolean-12-en-28-oic acid (4). Colorless wax. The ^{1}H-NMR, CDCl$_3$, δppm: 5.27 (1H, *t*, 3.5, H-12), 4.49 (1H, *dd*, 11.0, 6.0, H-3), 0.86 (3H, *s*, H-23), 0.85 (3H, *s*, H-24), 0.94 (3H, *s*, H-25), 0.75 (3H, *s*, H-26), 1.13 (3H, *s*, H-27), 0.90 (3H, *s*, H-29), 0.92 (3H, *s*, H-30), 2.29 (2H, *t*, 7.0, H-2’), 0.88 (3H, *t*, 7.0, H-21’). The ^{13}C-NMR, CDCl$_3$, δppm (Table 1).

3β-Pentadecanoyloxyolean-28(13)-lactone (5). White powder, mp. 241-243^{o}C (CHCl$_3$). The ^{1}H-NMR, CDCl$_3$, δppm: 4.48 (1H, *dd*, 10.0, 6.5, H-3), 0.85 (3H, *s*, H-23), 0.85 (3H, *s*, H-24), 0.89 (3H, *s*, H-25), 1.15 (3H, *s*, H-26), 1.04 (3H, *s*, H-27), 0.99 (3H, *s*, H-29), 0.86 (3H, *s*, H-30), 2.29 (2H, *t*, 7.0, H-2’), 0.88 (3H, H-15’). The ^{13}C-NMR, CDCl$_3$, δppm (Table 1).

Kaura-9(11),16-dien-18-oic acid (or grandiflorenic acid) (6). White crystal, mp. 155-157^{0}C (CHCl$_3$). The ^{1}H-NMR, CDCl$_3$, δppm: 5.23 (1H, *dd*, 3.5, 3.0, H-11), 4.91 (1H, *d*, 1.0, H-17a), 4.79 (1H, *s*, H-17b), 2.77 (1H, *brs*, H-13), 1.24 (3H, *s*, H-19), 1.02 (3H, *s*, H-20). The ^{13}C-NMR, CDCl$_3$, δppm (Table 1).

2-(3-Hydroxy-3,7,11,15-tetramethylhexadecyl)-3,5,6-trimethyl-1,4-dihydroxybenzene (7). Colorless oil. The ^{1}H-NMR, CDCl$_3$, δppm: 2.10 (3H, *s*, H-7), 2.15 (3H, *s*, H-8), 2.10 (3H, *s*, H-9), 1.22 (3H, *s*, H-20’), 0.87 (6H, *d*, 7.0, H-16’, H-17’), 0.56 (3H, *d*, 4.5, H-18’), 0.43 (3H, *d*, 4.5, H-19’). The ^{13}C-NMR, CDCl$_3$, δppm: 145.58 (C1), 117.37 (C2), 118.48 (C3), 144.55 (C4), 121.03 (C5), 122.63 (C6), 11.77 (C7), 12.20 (C8), 11.27 (C9), 20.77 (C1’), 31.58 (C2’), 74.53 (C3’), 39.84 (C4’), 21.05 (C5’), 37.44 (C6’), 32.72 (C7’), 37.49 (C8’), 24.46 (C9’), 37.30 (C10’), 32.81 (C11’), 37.47 (C12’), 24.81 (C13’), 39.39 (C14’), 27.99 (C15’), 22.63 (C16’), 22.72 (C17’), 19.67 (C18’), 19.76 (C19’), 23.81 (C20’).

Gramisterol (8). White crystal, mp. 162-163^{o}C (CHCl$_3$). The ^{1}H and ^{13}C-NMR data of **8** were good compatibility with the ones in literature[5]. The ^{1}H-NMR, CDCl$_3$, δppm: 5.17 (1H, *m*, H-7), 4.65 (1H, *d*, 1.0, H-28a), 4.71 (1H, *s*, H-28b), 0.53 (3H, *s*, H-18), 0.82 (3H, *s*, H-19), 0.95 (3H, *d*, 6.5, H-21), 1.03 (3H, *d*, 7.0, H-26), 1.02 (3H, *d*, 6.5, H-27), 0.98 (3H, *d*, 6.0, H-29). The ^{13}C-NMR, CDCl$_3$, δppm (Table 1).

Stigmasterol (9). White needle crystal, mp.164-166^{o}C (CHCl$_3$). The ^{1}H and ^{13}C-NMR data of **9** were good compatibility with the ones in literature[5]. The ^{1}H-NMR, CDCl$_3$, δppm: 5.34 (1H, *brs*, H-6), 5.16 (1H, *dd*, 15.0, 8.5, H-22), 5.02 (1H, *dd*, 15.0, 9.0, H-23), 3.51 (1H, *tt*, 11.0, 5.0, H-3), 1.01 (3H, *s*, H-18), 0.70 (3H, *s*, H-19), 1.03 (3H, *d*, 6.0, H-21), 0.79 (3H, *d*, 7.0, H-26), 0.84 (3H, *d*, 6.5, H-27), 0.81 (3H, *t*, 6.0, H-29). The ^{13}C-NMR, CDCl$_3$, δppm (Table 1).

β-Sitosterol 3-O-β-D-glucopyranoside (10). White powder, mp. 284-286^{0}C (MeOH). The

^{1}H and ^{13}C-NMR data of **10** were good compatibility with the ones in literature[4]. The ^{1}H-NMR, DMSO-d_6, δppm: 5.31 (1H, *brd*, H-6), 3.62 (1H, *m*, H-3), 4.83 (1H, *d*, 5.0, H-1'). The ^{13}C-NMR, DMSO-d_6, δppm (Table 1).

RESULTS AND DISCUSSION

Compound (**1**) was a triterpen with 30 carbons in the ^{13}C-NMR spectrum. **1** had an oxygenated carbon signal at δ 72.7 of C-3 as normal, which corresponded to an oxygenated methine proton signal at 3.73 ppm. Besides that it had 8 singlet methyl proton signals upfield at: 0.86, 0.93, 0.94, 0.96, 0.99, 0.99, 1.00 and 1.17. So **1** should be a friedelan triterpen by comparison with the published data.[7]

Compound (**2**) was also a triterpen with 30 carbons in the ^{13}C-NMR spectrum. **2** had an oxygenated methine carbon signal at δ 79.0 of C-3 as normal, one oxygenated methylene carbon signal at 69.7 ppm, 7 singlet methyl proton signals in high field zone of δ 0.79, 0.87, 0.88, 0.93, 0.94, 0.99 and 1.16 and two olefinic carbon signals at δ 122.4 and 142.2 of C-12 and C-13, respectively, in olean-12-ene skeleton. So **2** was determined as erythrodiol by comparison with the published data.[7]

Compound (**3**) was a white powder. The NMR spectra data of **3** had some signals like **2** such as a trisubstituted double bond (δ_H 5.19 *m*, δ_C 144.2 *s*, 122.3 *d*), an oxygenated methine group (δ_H 4.50, δ_C 80.6), an oxygenated methylene group (δ_H 3.54 and 3.21, δ_C 69.7) and 7 singlet methyl proton signals upfield at δ 0.86, 0.87, 0.87, 0.88, 0.94, 0.96 and 1.16. However, the hydroxyl group at C-3 was esterified to be -O-CO-R because the HMBC spectrum showed the correlation of proton H-3 (δ 4.50) with the resonant peak at δ 173.1 (C=O, C-1'). This spectrum also showed the correlation of proton H-2' with C-1'. Proton H-2' (2.29, 2H, *t*, 7.0 Hz) confirmed the presence of an acyl group -O-CO-R at C-3. The long side chain ester at C-3 was determined by MS. The ESI-MS (positive mode) showed a molecular ion peak at *m/z* = 688.5239 $[M+H_2O+NH_4]^+$ corresponding to the molecular formula of $C_{44}H_{76}O_3+H_2O+NH_4$ (Calcd. for $C_{44}H_{76}O_3+H_2O+NH_4$ 688.6244). The aglycone moiety with two hydroxyl groups had the mass of 441 amu ($C_{30}H_{49}O_2$) so the side chain moiety had the mass of 211 amu. This mass well suited to the alcanoyl group of -CO-$(CH_2)_{13}$-CH_3. So the compound was determined as 28-hydroxy-3β-tetradecanoyloxyolean-12-ene or 3β-tetradecanoyloxyerythrodiol via the comparison with the literature.[2]

Compound (**4**) was a colorless wax. The NMR spectra data of **4** had some signals like **3** such as a trisubstituted double bond (δ_H 5.27 *m*, δ_C 143.6 *s*, 122.6 *d*), an oxygenated methine group (δ_H 4.49, δ_C 80.6), 8 singlet methyl proton signals in high field zone and an acyl group -O-CO-R at C-3 via the correlation between H-3 and the resonant peak at δ 173.7 (C=O, C-1'). However, **4** had one more carboxyl carbon signal at δ 184.0 of C-28 by the correlations of H-18, H-16, H-22 with C-28. The long side chain ester at C-3 was determined by MS. The ESI-MS (positive mode) showed a molecular ion peak at *m/z* = 803.5495 $[M+K]^+$ corresponding to the formula of $C_{51}H_{88}O_4$+K (Calcd. for $C_{51}H_{88}O_4$+K 803.6320), from that, the alcanoyl group shoud be $-CO-(CH_2)_{19}-CH_3$. So the compound was determined as 3β-henicosanoyloxyolean-12-en-28-oic acid via the comparison with the literature.[2]

Compound (**5**) was a white powder. The NMR spectra data of **5** had some signals like **3** and **4** such as an oxygenated methine group (δ_{H-3} 4.48, δ_{C-3} 80.4), 8 singlet methyl proton signals in high field zone and an acyl group -O-CO-R at C-3 via the correlation between H-3 and the resonant peak at δ 173.7 (C=O, C-1'). However, **5** had one more carboxyl carbon at δ 180.3 (-COO-, C-28) and one more oxygenated quaternary carbon at δ 91.8 (>C-O, C-13) instead of two olefinic carbon signals of C-12 and C-13 as normal. Besides that, the IR spectrum showed a strong absorption at ν 1755 cm^{-1} of C=O (lactone), so the olefinic carbon at C-13 changed to the oxygenated carbon and created a lactone ring with the carboxyl group (C-28). This was confirmed by the correlation signals of H-11, H-12 and H-18 with a resonant peak at δ 91.8 (C-13) and the correlation signals of H-16 with a resonant peak at δ 180.3 (C-28). The long side chain ester at C-3 was determined by MS. The ESI-MS showed a molecular ion peak at *m/z* = 716.5585 $[M+H_2O+NH_4]^+$ corresponding to the molecular formula of $C_{45}H_{76}O_4+H_2O+NH_4$ (Calcd for $C_{45}H_{76}O_4+H_2O+NH_4$ 716.6139), so the alcanoyl group shoud be $-CO-(CH_2)_{13}-CH_3$. Moreover, the comparison of ^{13}C-NMR data of the aglycone of **5** with those of literature[3] showed good compatibility. So this compound was determined as 3β-pentadecanoyloxyolean-28(13)-lactone.

Table 1. NMR data of compounds (1), (2), (3), (4), (5), (6), (8), (9) and (10)

N^0	1	2	3	4	5	6	8	9	10
1	15.83	38.64	38.30	38.10	38.63	40.79	37.05	37.29	36.80
2	35.25	27.25	23.60	23.59	23.65	20.16	31.12	28.92	29.23
3	72.78	79.02	80.57	80.60	80.40	38.27	76.23	71.82	70.07
4	49.22	38.80	37.78	37.76	37.84	44.76	40.29	39.81	39.33
5	37.88	55.21	55.28	55.34	55.09	46.64	46.71	140.79	140.10
6	41.78	18.38	18.26	18.20	17.59	18.48	26.68	121.71	121.19
7	17.58	32.62	32.55	32.58	33.48	29.70	117.53	31.70	31.34
8	53.24	39.82	39.84	39.32	42.20	42.28	139.09	31.99	31.34
9	37.15	47.62	47.53	47.58	50.59	155.97	49.69	50.21	49.58
10	61.41	36.96	36.85	37.02	36.78	38.82	34.87	36.54	36.17

11	35.60	23.60	23.55	23.42	31.49	114.92	21.40	21.10	20.56
12	30.67	122.39	122.30	122.58	18.18	37.93	39.59	39.72	38.29
13	39.71	144.24	144.25	143.62	**91.76**	41.27	43.42	42.25	41.82
14	38.41	41.76	41.75	40.97	42.25	44.97	54.98	56.90	56.15
15	32.87	25.59	25.57	27.70	26.53	50.34	22.93	24.38	23.82
16	36.12	22.06	22.04	22.91	20.85	158.54	27.96	28.26	27.74
17	30.06	36.96	36.97	46.57	44.05	105.47	56.05	56.01	55.41
18	42.88	42.37	42.38	41.60	50.37	184.33	11.86	19.40	11.62
19	35.37	46.51	46.46	45.88	37.45	28.25	14.14	12.01	19.04
20	28.20	31.05	30.96	30.67	31.35	23.60	36.20	40.48	35.44
21	32.38	34.12	34.12	33.83	34.18		18.86	21.10	18.57
22	39.31	31.10	31.06	32.47	31.90		34.70	138.31	33.33
23	11.63	28.11	28.07	28.08	27.92		31.01	129.32	25.45
24	16.41	15.59	16.76	16.73	16.49		156.84	51.26	45.13
25	18.27	15.52	15.57	15.38	16.04		33.85	31.94	28.69
26	20.13	16.76	16.77	17.19	18.32		21.88	19.02	18.90
27	18.65	25.95	25.92	25.92	19.60		22.01	21.23	19.65
28	32.12	69.70	69.71	183.99	180.29		105.99	25.41	22.59
29	35.04	33.20	33.19	33.06	33.28		15.16	12.31	11.74
30	31.82	23.60	23.60	25.59	23.87				
1’			173.70	173.72	173.69				100.79
2’			34.88	34.87	34.83				73.43
3’			14.11*	14.11*	14.09*				76.74
4’									76.69
5’									76.93
6’									61.06

*: terminus methyl groups

Compound (**6**) was a diterpen with 20 carbons in the ^{13}C-NMR spectrum. It had four olefinic carbon signals of two olefinic bonds at 158.5 (=**C**<, C-16) and 105.5 (=**C**H$_2$, C-17); 155.9 (=**C**<, C-9) and 114.9 (=**C**H-, C-11) of a kauran skeleton, one carboxyl carbon signal of a –COOH group at δ 184.3 and three singlet methyl carbon signals in high field zone. So **6** was determined as kaura-9(11),16-dien-18-oic acid (or grandiflorenic acid) by comparison with the published data.[6]

CONCLUSION

From flower of *Wedelia trilobata* Hitch. **five triterpenoids**: friedelan-3β-ol (or epifriedelanol) **(1)**, erythrodiol **(2),** 28-hydroxy-3β-tetradecanoyloxyolean-12-ene (or 3β-tetradecanoyloxyerythrodiol) **(3)**, 3β-henicosanoyloxyolean-12-en-28-oic acid **(4)**, 3β-pentadecanoyloxyolean-28(13)-lactone **(5), one diterpenoid**: kaura-9(11),16-dien-18-oic acid (or grandiflorenic acid) **(6)**, **one hydroquinone derivative**: 2-(3-hydroxy-3,7,11,15-tetramethylhexadecyl)-3,5,6-trimethyl-1,4-dihydroxybenzene (or tocopherolhydroquinone) **(7)** and **three sterols**: gramisterol **(8),** stigmasterol **(9)** and β-sitosterol 3-*O*-β-D-glucopyranoside **(10)** were isolated. Among them, **2, 5, 7, 8** were found for the first time in *Wedelia* species.

MỘT SỐ HỢP CHẤT TỪ HOA CÂY SƠN CÚC BA THÙY *WEDELIA TRILOBATA* (L.) HITCH., HỌ CÚC (ASTERACEAE)

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TÓM TẮT: *Cây Sơn cúc ba thùy, Wedelia trilobata Hitch., chưa được nghiên cứu nhiều trên thế giới cũng như ở Việt Nam. Từ hoa cây Sơn cúc ba thùy, mười hợp chất đã được cô lập: friedelan-3β-ol (hoặc epifriedelanol) (1), erythrodiol (2), 28-hydroxy-3β-tetradecanoyloxyolean-12-en (hoặc 3β-tetradecanoyloxyerythrodiol) (3), acid 3β-henicosanoyloxyolean-12-en-28-oic (4), 3β-pentadecanoyloxyolean-28(13)-lacton (5), acid kaura-9(11),16-dien-18-oic (hoặc acid grandiflorenic) (6), 2-(3-hydroxy-3,7,11,15-tetrametylhexadecyl)-3,5,6-trimetyl-1,4-dihydroxybenzen (hoặc tocopherolhydroquinon) (7), gramisterol (8), stigmasterol (9) and β-sitosterol 3-O-β-D-glucopyranosid (10). Cấu trúc của những hợp chất này được xác định dựa trên dữ liệu phổ NMR và kết hợp với so sánh tài liệu tham khảo.*

Từ khóa: *Họ Cúc, Wedelia trilobata, triterpen, diterpen, sterol.*

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