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Phytochemical Studies of *Ficus deltoidea* var *kunstleri*

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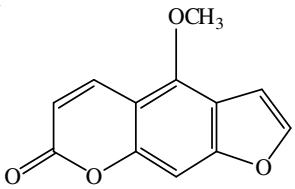
Ficus deltoidea or locally known as Mas Cotek is one of the traditional medicinal plants. The aim of this work is to determine the chemical structure of isolated compounds from *F. deltoidea* var. *kunstleri* by using various spectroscopy and also by comparison with previous literature data. Chromatographic purification of hexane and dichloromethane crude extract of *F. deltoidea* var. *kunstleri* (leaves and stem barks) yielded four pure compounds *viz.*, α-amyrin (**1**), lupeol (**2**) bergapten (**3**) and β-amyrin cinnamate (**4**) along with four mixtures; mixture of sesquiterpenes: tanacetene (**5**) and β-elemene (**6**), mixture of sterols; stigmasterol (**7**) and β-sitosterol (**8**), a mixture of triterpenes: lupeol, α,β-amyrin (**9**), lupenone, α,β-amyrenone (**10**). These compounds were identified through analysis mainly 1D and 2D NMR and also by comparison with previous studies. Even though **1, 3, 4, 5, 6, 7** and **10** are known compounds, but our best of knowledge these compounds are first time reported from this species.

Keywords: *Ficus deltoidea* var. *kunstleri*, Triterpenes, Sesquiterpenes, Sterols.

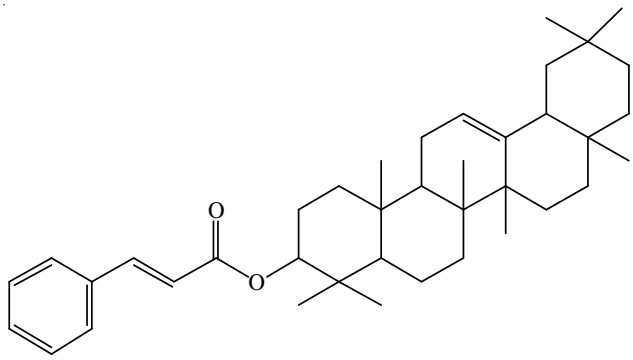
INTRODUCTION

The family *Moraceae* is in the major group of Angiosperms which comprising 40 genera and more than 1000 species. It is evergreen or shrub that can be found in tropical and subtropical regions. The plant of *Moraceae* contains a milky latex and has alternate or opposite leaves and small petalless male or female flowers. Ficus is one of the genus of *Moraceae.* It comprises a total of 900 species. One of its species, *Ficus deltoidea* is commonly known as Mas Cotek, Serapat Angin, Telinga Beruk or Sempit-sempit in Malaysia due to fine spots with gold colour on the surface of each leaves [1] and also known as Kangkalibang by African which can be recognized by very distinctive inflorescence. It has fruits with seed like achene and usually enclosed within a syncarp which developed from an enlarged hollow fleshy receptacle. It is distributed in Malesia that includes Thailand, Indonesia and Malaysia [2]. Leaves and figs of the *F. deltoidea* have been studied and were found to contain diverse compounds, including flavonoids and terpenes [3-7]. Moreover, intensive pharmacological investigation revealed its significant biological properties, such as antidiabetic agent [8-12] and antioxidant [9,13-15].

Leaves and stem barks of *F. deltoidea* var. *kunstleri*. However, it has not been widely phytochemically investigated so far. Therefore, the present study is to search for bioactive compounds from its hexane crude extract from the air-dried leaves and stems of *F. deltoidea* var *kunstleri* and lead to the isolation of four pure compounds *e.g.*, α-amyrin (**1**), lupeol (**2**) bergapten (**3**) and β-amyrin cinnamate (**4**) along with four mixtures; mixture of sesquiterpenes: tanacetene (**5**) and β-elemene (**6**), mixture of sterols; stigmasterol (**7**) and β-sitosterol (**8**), a mixture of triterpenes, lupeol, α,β-amyrin (**9**), lupenone, α,β-amyrenone (**10**). All the spectroscopic data are in good agreement with the reported literature values [4,16-22].



Structure of bergapten (**3**)



Structure of β-amyrin cinnamate (**4**)

EXPERIMENTAL

The leaves and stem barks of *Ficus deltoidea* var *kunstleri* was selected for the study. It was collected from Kuala Selangor in October 2014. A voucher specimen was deposited at herbarium of Chemistry Department, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris, Malaysia.

Extraction and isolation: Air-dried ground leaves and stem barks of *F. deltoidea* var. *kunstleri* (2 kg) were macerated sequentially with hexane, dichloromethane and methanol for three cycles at room temperature. The extracts were dried under high pressure. The extraction gave: 3.63, 2.15 and 3.55 % for hexane, dichloromethane and methanol crude extracts, respectively.

The brown syrup (43.9 g) hexane crude extract was fractionated on normal phase silica gel (0.06-0.02 mm) open-column chromatography, eluting with hexane-DCM-MeOH (1:0:0 to 0:0:1). A total of 130 collections were made and pooled into 45 major fractions (A1-A45) based on their TLC profiles. Subfraction A2 was further fractionated on a silica gel column (0.04-0.063 mm) to yield a mixture of **5** and **6** (9.6 mg) while A31 was rechromatographed over PTLC, with hexane-DCM (2:8 v/v) as eluent afforded mixture of **7** and **8** (3.6 mg). Fraction labelled as A13 and A27 were separately rechromatographed over PTLC, eluting with hexane-CH_2Cl_2 (v/v): (5:5), (6:4), (1:9) and (15:85) to afford **4** (32.8 mg) and **9** (30.6 mg), respectively. While, another subfraction A20 was further fractionated on silica gel column hexane-CH_2Cl_2 (7:3 v/v) to afford **10** (15.2 mg). The dichloromethane (DCM) extract (27 g) was loaded to silica gel CC, eluting with hexane-DCM-methanol (1:0 to 0:1 v/v), to give 26 major fractions (B1-B26). Rechromatography of B15 and B16 over silica gel using hexane-CH_2Cl_2 (2:8 v/v) and further purify by using PTLC with CH_2Cl_2 as solvent system to afford furanocoumarins; **3.** Compound **1** and **2** was purified from fraction A23 by using PTLC.

Bergapten (3): ^{1}H NMR (500 MHz, $CDCl_3$), δ: 6.27 (*d, J* = 9.75 Hz, H-3), 8.16 (*d, J* = 9.75 Hz, H-4), 7.13 (*s,* H-8), 7.59 (*d, J* = 2.3Hz, H-2'), 7.02 (*d*, *J* = 2.3 Hz, H-3') and 4.27 (*s,* 5-OCH_3).^{13}C NMR (125 MHz, $CDCl_3$), δ: 161.4 (C-2), 112.6 (C-3), 139.4 (C-4), 149.5 (C-5), 112.7 (C-6), 158.3 (C-7), 93.9 (C-8), 152.8 (C-9), 106.4 (C-10), 144.9 (C-2'), 105.1 (C-3'), 60.2 (5-OCH_3).

β-Amyrin cinnamate (4): ^{1}H NMR (500 MHz, $CDCl_3$), δ: 1.58 (1H, *s*, H-1), 0.80 (1H, *s*, H-1), 1.66 (2H, *m*, H-2), 4.65 (1H, *t*, *J* = 8.6 Hz, H-3), 0.80 (1H, *s*, H-5), 1.46 (1H, *m*, H-6), 1.32 (1H, *m*, H-7), 1.47 (1H, *m*, H-7), 1.54 (1H, *s*, H-9), 1.87 (2H, *m*, H-11), 5.18 (1H, *t*, *J* = 3.4 Hz, H-12), 1.08 (1H, *s*, H-15), 1.66 (1H, *s*, H-15), 1.94 (1H, *m*, H-16), 1.88(1H, *s*, H-18), 0.95 (1H, *s*, H-19), 1.60 (1H, *s*, H-19), 1.10(1H, *m*, H-21), 1.30(1H, *m*, H-21), 1.20 (1H, *t*, H-22), 1.39 (1H, *s*, H-22), 0.98 (3H, *s*, H-23), 0.92 (3H, *s*, H-24), 0.92 (3H, *s*, H-25), 0.87 (1H, *d*, *J* = 2.3 Hz H-26), 1.14 (3H, *s*, H-27), 0.83 (3H, *s*, H-28), 0.83 (3H, *s*, H-29), 0.87 (3H, *s*, H-30), 7.52 (1H, *d*, *J* = 4 Hz, H-2'), 7.39 (1H, *d*, *J* = 1.8 Hz,, H-3'), 7.37 (1H, *s*, H-4'), 7.38 (1H, *d*, *J* = 2.3 Hz, H-5'), 7.53 (1H, *d*, *J* = 4 Hz, H-6'), 7.66 (1H, *d*, *J* = 16 Hz, H-7'), 6.45 (1H, *d*, *J* = 16 Hz, H-8'). ^{13}C NMR (125 MHz, $CDCl_3$), δ: 38.3 (C-1), 23.6 (C-2), 81.1 (C-3), 38.0 (C-4), 55.3 (C-5), 18.4 (C-6), 32.6 (C-7), 39.9 (C-8), 47.6 (C-9), 36.9 (C-10), 23.6 (C-11), 121.7 (C-12), 145.3 (C-13), 41.8 (C-14), 26.2 (C-15), 26.9 (C-16), 32.7 (C-17), 47.3 (C-18), 46.8 (C-19), 31.2 (C-20), 34.8 (C-21), 37.2 (C-22), 28.2 (C-23), 16.9 (C-24), 15.6 (C-25), 16.8 (C-26), 26.1 (C-27), 28.5 (C-28), 33.4 (C-29), 23.7 (C-30), 134.6 (C-1'), 128.1 (C-2'), 128.9 (C-3'), 130.2 (C-4'), 128.9 (C-5'), 128.1 (C-6'), 144.4 (C-7'), 118.9 (C-8'), 166.9 (C-9').

RESULTS AND DISCUSSION

Bergapten (**3**) has been isolated as yellow solid from dichloromethane extract. The molecular formula of bergapten is $C_{12}H_8O_4$. From the molecular formula indicated the presence of nine units of unsaturation and the LCMS spectrum revealed a protonated molecule ion peak at 217.00 $[M+H]^+$.

The structural elucidation of **3** was carried out by means of NMR (^{1}H, ^{13}C, COSY, HMQC and HMBC). The ^{1}H NMR spectra of compound revealed downfield signals at δ 7.59 (*d, J* = 2.3 Hz), 7.13 (*s*), 7.02 (*d, J* = 2.3 Hz) with a pair of *cis* configuration at 8.16 (*d, J* = 9.75 Hz) and 6.27 (*d, J* = 9.75 Hz). A methoxy group also has been identified from the spectrum at signal δ 4.27 (*s*).

The furanocoumarins was directly inferred from its ^{13}C NMR spectrum with the aid of a DEPT experiment which exhibited 12 signals comprising five non-protonated carbon; δ158.3 (C-7), 152.8 (C-9), 149.5 (C-5), 112.6 (C-3) and 106.4 (C-10) with one carbonyl group at δ 161.4 (C-2); five methine carbons; δ 144.9 (C-2'), 139.4 (C-4), 112.6 9C-3), 105.1 (C-3'), 93.9 (C-8) and one methoxy carbon at δ 60.2 ppm (5-OCH_3).

Fragments of C3-C4 and C2'-C3' were analyzed from proton-coupled spectrum. Signal at δ 93.9 ppm was assigned as C-8 and its position has been confirmed by corroboration with the HMBC spectrum which displayed correlation of C-8 proton at 7.13 (*s*) with carbon resonance δ 152.8, 106.4, 112.7 and 158.4 assigned for C-9, C-10, C-6 and C-7 respectively.

Identification of compound **3** also supported with UV experiment which showed λ_{max} at 223, 243, 249, 259, 268 and 311 nm [23]. The IR spectrum showed the presence of carbonyl group at 1726 cm^{-1}, methoxy group at 2820 and 1550 cm^{-1} for aromatic ring.

The complete assignment for the proton and carbon signals are shown in Table-1. These data indicated that the compound **3** is a furanocoumarins; bergapten as characteristic signals unit were noticeable in both the NMR spectra. The identification of compound **3** as bergapten was confirmed by comparison of empirical data with published values [22].

Identification of compound **4** was supported by the analysis of ^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H COSY, DEPT, HMQC, HMBC and MS spectra. Compound **4** was obtained as white amorphous from hexane and DCM crude extract. The MS spectrum revealed a protonated molecule ion peak at 557.40 $[M+H]^+$ corresponding to its molecular formula as $C_{39}H_{57}O_2$ thus, implying 12 degrees of unsaturation.

IR spectral data suggested the following information for the structural elucidation of compound **4**: 1730 cm^{-1} (ester carbonyl), 1644 cm^{-1} (olifinic carbon) and 1553 cm^{-1} (aromatic ring). While the maximum absorption of UV spectra for **4** are 231, 235, 239 and 264 nm.

TABLE-1

Position	δ_H (*J* Hz) (ppm)	δ_C (ppm)
2		161.4
3	6.27 (*d, J* = 9.75 Hz)	112.6
4	8.16 (*d, J* = 9.75 Hz)	139.4
5		149.5
6		112.7
7		158.3
8	7.13 (*s*)	93.9
9		152.8
10		106.4
2'	7.59 (*d, J* = 2.3 Hz)	144.9
3'	7.02 (*d*, *J* = 2.3 Hz)	105.1
5-OCH_3	4.27 (*s*)	60.2

Through ^{1}H NMR spectra of **4** in $CDCl_3$ showed signals δ 7.66 (*d*, *J* = 16 Hz) and 6.45 (*d*, *J* = 16 Hz) that belongs to olefinic proton for H-7' and H-8', respectively. This two olefinic protons is coupled to each other which also established in COSY analysis. In addition, ^{1}H NMR spectrum revealed the presence of eight tertiary methyl; δ 0.83 (H-28 and H-29), 0.87 (H-30), 0.92 (H-24 and H-25), 0.98 (H-23) and 1.14 (H-27). The aromatic protons were observed at δ 7.52 (*d*, *J* = 4 Hz, H-2'), δ 7.39 (*d*, *J* = 1.8 Hz, H-3'), δ 7.37 (*s*, H-4'), δ 7.38 (*d*, *J* = 2.3 Hz, H-5') and δ 7.53 (*d*, *J* = 4 Hz, H-6'), indicating the existing of cinnamate moiety in this compound. The signal presence at δ_H 5.18 belongs to methine proton of C-12.

The ^{13}C NMR spectrum of compound **4** suggesting a presence of one carbonyl carbon at δ166.9 (C-9') and five double bond carbons at δ 118.9 (C-8'), 121.7 (C-12), 128.1 (C-2' and C-6'), 128.9 (C-3' and C-5'), 130.2 (C-4'), 134.6(C-1'), 144.4 (C-7') and 145.3 (C-13) while DEPT NMR spectrum showed the presence of 39 signals corresponding to 39 carbons which consist of nine quaternary carbons : δ 38.0 (C-4), 39.9 (C-8), 36.9 (C-10), 45.3 (C-13), 41.8 (C-14), 32.7 (C-17), 31.2 (C-20), 134.6 (C-1') and 166.9 (C-9'); 13 methine carbons : δ 81.1 (C-3), 55.3 (C-5), 47.6 (C-9), 121.7 (C-12), 47.3 (C-18),128.1 (C-2'), 128.9 (C-3'), 130.2 (C-4'), 128.9 (C-5'), 128.1 (C-6'), 144.4 (C-7') and 118.9 (C-8'); ten methylene carbons : δ 38.3 (C-1), 27.0 (C-2),18.4 (C-6), 32.6 (C-7), 23.6 (C-11), 26.9 (C-16), 46.8 (C-19) and 37.2 (C-22) and eight methyl carbons: δ 28.2 (C-23), 15.6 (C-24), 15.6 (C-25), 16.8 (C-26), 26.1 (C-27), 28.5 (C-28), 33.4 (C-29) and 23.7 (C-30), Thus, this compound has one cinnamate group and its C-skeleton contains 30 carbons. ^{1}H and ^{13}C NMR data of compound **4** showed similarities β-amyrin, except for some notable differences for H-3 and C-3 values which appeared more downfield due to presence of cinnamate moiety. The C-3 signal of **4** was observed at lower field (δ 81.3) while, for β-amyrin at δ 79.0.

In the HMBC spectral anaylsis, the cross peaks between H-3 and C-9' showed a linkage between methine proton of β-amyrinto its derivatives; the carbonyl ester carbon. Futhermore, proton signal of H-7' to C-9', H-8' to C-9', supported the position of C-9'. In addition, the HMBC correlation between H-3 (δ_H 4.66) and C-1,C-2, C-4 and C-24; between H-1 (δ_H 1.61) and C-3 and tertiary methyl signals H-23 (δ_H 0.98) and C-3 firmly established and supported the position of C-3. The ^{1}H-^{1}H COSY spectrum established suggested fragments C-5'-C-6'-C2'-C3' and C-15-C-16.

The complete assignment for the proton and carbon signals were tabulated in Table-2. These data indicated that compound **4** is a derivative of β-amyrin as characteristic signals of a cinnamate unit were noticeable in both the ^{1}H and ^{13}C NMR spectra. Finally, the identification of compound **4** as β-amyrin-cinnamate proven by complete assignments of ^{1}H and ^{13}C which was isolated compound from shea tree [22].

TABLE-2

Position	δ_H (*J* Hz) (ppm)	δ_C (ppm)
1	1.58 (*s*), 0.80 (*s*)	38.3
2	1.66 (*m*)	23.6
3	4.65 (*t*, *J* = 8.6 Hz)	81.1
4		38.0
5	0.80 (*s*)	55.3
6	1.46 (*m*)	18.4
7	1.32 (*m*), 1.47 (*m*)	32.6
8		39.9
9	1.54 (*s*)	47.6
10		38.0
11	1.87 (*m*)	23.6
12	5.18 (*t*, *J* = 3.4 Hz)	121.7
13		145.3
14		41.8
15	1.08 (*m*), 1.66 (*m*)	26.2
16	1.94 (*m*)	26.9
17		32.7
18	1.88 (*m*)	47.3
19	0.95 (*m*), 1.60 (*m*)	46.8
20		31.2
21	1.10 (*m*), 1.30 (*m*)	34.8
22	1.20 (*m*), 1.39 (*m*)	37.2
23	0.98 (*s*)	28.2
24	0.92 (*s*)	16.9
25	0.92 (*s*)	15.6
26	0.87 (*d*, *J* = 2.3 Hz)	16.8
27	1.14 (*s*)	26.1
28	0.83 (*s*)	28.5
29	0.83 (*s*)	33.4
30	0.87 (*s*)	23.7
1'		134.6
2'	7.52 (*d*, *J* = 4 Hz)	128.1
3'	7.39 (*d*, *J* = 1.8 Hz)	128.9
4'	7.37 (*s*)	130.2
5'	7.38 (*d*, *J* = 2.3 Hz)	128.9
6'	7.53 (*d*, *J* = 4 Hz)	128.1
7'	7.66 (*d*, *J* = 16 Hz)	144.4
8'	6.45 (*d*, *J* = 16 Hz)	118.9
9'		166.9

Conclusion

Study for bioactive compounds from *F. deltoidea* var *kunstleri* led to isolation of four pure compounds *i.e.*, β-amyrin (**1**), lupeol (**2**) bergapten (**3**) and β-amyrin cinnamate (**4**) along with four mixtures; mixture of sesquiterpenes: tanacetene (**5**) and β-elemene (**6**), mixture of sterols; stigmasterol (**7**) and β-sitosterol (**8**), a mixture of triterpenes: lupeol α,β-amyrin (**9**), lupenone, α,β-amyrenone (**10**). These compounds were identified through analysis mainly 1D and 2D NMR and also by comparison with previous studies. Even though **1, 3, 4, 5, 6, 7** and **10** are known compounds, these compounds are first time reported from this species.

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