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].
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 0:1:0) as eluent to afford a mixture of A31 was rechromatographed over PTLC, with hexane-DCM (0.04-0.063 mm) to yield a mixture of A1 and A27. Fraction labelled as A1, A2 and A3 were separately rechromatographed on silica gel column hexane-CH$_2$Cl$_2$ (2:8 v/v) as eluent to afford mixture of 26 major fractions (B1-B26). Rechromatography of B15 and B16 over silica gel using hexane-CH$_2$Cl$_2$ (2:8 v/v) and further purified by using PTLC with CH$_3$Cl$_2$ as solvent system to afford furanocoumarins; 3, Compound 1 and 2 was purified from fraction A23 by using PTLC.

**Bergapten (3):** 1H 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, $J = 2.3$ Hz, H-2), 7.02 (d, $J = 2.3$ Hz, H-3') and 4.27 (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$_2$Cl$_2$ (v/v): (5:5), (7:3 v/v) 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$_2$Cl$_2$ (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$_2$Cl$_2$ (2:8 v/v) and further purified by using PTLC with CH$_3$Cl$_2$ as solvent system to afford furanocoumarins; 3, Compound 1 and 2 was purified from fraction A23 by using PTLC.

**RESULTS AND DISCUSSION**

Bergapten (3) has been isolated as yellow solid from dichloromethane extract. The molecular formula of bergapten is C$_{20}$H$_{18}$O$_{5}$. 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 (1H, 13C, COSY, HMOC and HMBC). The 1H 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) and 6.27 (d, $J = 9.75$ Hz). The methoxy group also has been identified from the spectrum at signal δ 6.47 (s).

The furanocoumarins was directly inferred from its 13C NMR spectrum with the aid of a DEPT experiment which showed 12 signals comprising five non-protonated carbon; δ18.3 (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-4); five methine carbons; δ 144.9 (C-2'), 139.4 (C-4), 126.2 (C-6) and 93.9 (C-8) and one methoxy carbon at δ 60.2 (δ-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 correlation with the HMBC spectrum which displayed correlation of C-8 proton at δ 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 λmax at 233, 243, 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 also supported with UV experiment which showed λmax at 233, 243, 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.

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.
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 1H and 13C NMR spectra. Finally, the identification of compound 4 as β-amyrin-cinnamate proven by complete assignments of 1H and 13C which was isolated compound from sheatree [22].

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: tancatetene (5) and β-elemene (6), mixture of sterols; stigmasterol (7) and β-sitosterol (8), a mixture of triterpenes: lupeol αβ-amyrin (9), lupenone, αβ-amyrone (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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REFERENCES