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# Phytochemical investigation and biological studies of Euphorbia tithymaloides L. family Euphorbiaceae

**Sara M. Foda[1], Mona G. Zaghloul [2], Amani M. Marzouk [1' 2], El-Sayed Shaker Mansour [2]**

*[1]Department of Pharmacognosy, Faculty of Pharmacy, Delta University for Science and Technology,*
*[2]Department of Pharmacognosy, Faculty of Pharmacy, Mansoura University*

*** Correspondence:** Amani M. Marzouk; [Faculty of pharmacy, Delta University for Science and Technology, Gamasa, Egypt]; Tel [+20102654881]; Email: amarzouk2003@yahoo.co.uk

**ABSTRACT**
Phytochemical investigation of the petroleum ether and methylene chloride extracts of the stems of *Euphorbia tithymaloides* L. (Euphorbiaceae) afforded the triterpenes friedelane-3β-ol, 3-oxo-friedelane, euphane-7, 24-diene, 3β-ol (butyrospermol), and euphane -7, 25-diene, 3, 24-β- diols in addition to the diterpene derivative 1 α, 13 β, 14 α-trihydroxy-3 β, 7 β-dibenzenzoyloxy-9 β, 15 β-diacetoxyjatropha-5, 11-*E*-diene and the phytosterol β-sitosterol. The structures of the isolated compounds were established depend on spectroscopic and chemical evidences. The petroleum ether, methylene chloride, and methanolic extracts of the stems of *E. tithymaliodes* L. were tested *in vitro* by determining their action on oxidative stress status of rat liver homogenate for their hepatoprotective activity in addition, the pet ether extract was tested *in vivo* for its hepatoprotective activity and diterpene derivative was tested *in vitro* for cytotoxic activity.

***Keywords:*** *Euphorbia tithymaloides, triterpenes, diterpenes, Hepatoprotective activity, Cytotoxic activity.*

## 1. Introduction

Family Euphorbiaceae is a large family with three hundred genera and around eight thousand species (Webster, 1994). The genus *Euphorbia* is a major genus in the family including about two thousand species ranging from annuals to trees (Davis, 1970). *Euphorbia tithymaloides* L. (syn. *Pedilanthus tithymaloides* (L.) Poit.); devil's-backbone or redbird flower is a persistent juicy spurge, the shrub is native to tropical, Northern and Central Americas (Spoerke and Smolinske, 1990; Rajani and Neetu, 2019). Its fresh leaves are used for treating cuts, wounds and reported to have anthelmintic (Kumar *et al.*, 2015), anti-diabetic ((Farheen and Ramesh, 2016; Wandita *et al.*, 2018), anticancer (Pettit *et al.*, 2002), anti-filarial (Kamalakannan *et al.*, 2010) and analgesic activities (Ghosh *et al.*, 2013). A few phytochemical surveys on *E. tithymaloides* reported the isolation of long-chain alcohols, sterols, terpenes as cycloartenol triterpenes (Mongkolvist and Sutthivaiyakit, 2007), flavonoids as Kaempferol and isoquercitrin; flavonoid glycosides as quercetin; phenols as gallic acid; resins and coumarins as scopoletin (Abreu *et al.*, 2008). Euphorbia tithymaliodes has never been assessed for hepatoprotective activity of its extracts. In this study, we describe the separation and naming of 6 compounds from petroleum ether and methylene chloride extracts of *Euphorbia tithymaliodes* L. stems. Different extracts were tested *in vitro* and/or *in vivo* for their hepatoprotective action which measured by their influence on oxidative stress status of rat liver. The diterpene (1 α, 13 β, 14 α-trihydroxy-3 β, 7 β-dibenzenzoyloxy-9 β, 15 β-diacetoxyjatropha-5, 11-*E*-diene) was tested for cytotoxic activity.

## 2. Material and methods

*2.1. Plant material*

Stems of *Euphorbia tithymaliodes* L. were gathered from plants growing on Delta University for Science and Technology campus, Egypt in April 2017. The plant name was kindly established by Horticulture Department staff members, Faculty of Agriculture, Mansoura University, Egypt. A voucher sample was placed at the Department of Pharmacognosy, Faculty of Pharmacy, Mansoura University. At room temperature, the collected freshly stems of *E. tithymaliodes* were air-dried and milled.

*2.2. General experimental procedures*

A rotary flash evaporator (Bauchi, Switzerland) was used. A Shimadzu FTIR-8100 spectrometer (Shimadzu, Japan) was used for recording IR bands. Nuclear Magnetic Resonance spectra ($^1$H- NMR, APT, HMBC and NOSEY spectra) were recorded on Bruker dpx-400 spectrometer (Bruker, Germany) at 400 MHz for $^1$H-NMR and 100 MHz for $^{13}$C-NMR and JOEL ECA-500 II spectrometer (JOEL, Japan) at 500 MHz for $^1$H-NMR and 125 MHz for $^{13}$C-NMR. A Shimadzu Qp-2010 plus mass spectrometer (Shimadzu, Japan) was used for recording EI mass spectra (70 eV). Melting points (uncorr.) were documented on Yamagimoto micro-melting point apparatus MP-500D (Japan). Puriflash 4100 system consisting of a mixing of HPLC quaternary pump and flash purification into same device. A PDA-UV-V is detector 190-840 nm, a fraction collector, and a sample loading module, flow rate up to 250 ml/min, a maximum pressure of 100 bars. Prep HPLC column was used with inner diameter range from 50 to 100 mm and 250 to 1000 mm in length. Normal phase silica was used for preparative HPLC purification of compounds, column used normal phase: 20-100 mg. Interchim Software 5.0 was used for process monitoring and system controlling (Interchim; Montlucon, France). Thermostatic water bath YCW-01 (22 L) (Taiwan). PH meter AD8000 (Romania). Vortex mixer (230V-50/60HZ) (Korea). Centrifuge (PLC-012) (Taiwan). Spectrophotometer (JENWAY, model 7305, UK). Silica gel 60-230 mesh was performed for Colum chromatography (Merck, Germany). Thin Layer Chromatography was performed on precoated TLC plates with silica gel 60 $GF_{254}$ (20 x 20 cm x 0.2 mm thick) on aluminum or plastic sheets (Merck, Germany). Developed chromatograms were imagined by spraying with 0.01 % vanillin/$H_2So_4$, then heating until the spot color reached maximum development. Thiobarbituric acid (TBA) was obtained from Sigma (UK). Kits which were obtained from Biodiagnostics (Egypt), used for measuring the liver enzymes. Silymarin (SEDICO Pharmaceutical Company, Egypt). Sodium dodecyl sulfate (SDS), Acetic acid 99 % (v/v), Potassium chloride, N-butanol, Sodium hydroxide (El-Nasr Company for Pharmaceutical Chemicals, Egypt). Cell lines which were obtained from ATCC via a Holding company for biological products and vaccines (VACSERA, Egypt), used for cytotoxicity assay. [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide] (MTT), DMSO (dimethyl sulfoxide), doxorubicin and RPMI-1640 medium (Sigma, USA). Ferric chloride (Chemajet chemical company, Egypt). Ascorbic acid (Oxford Lab Chem, India). Phosphate-buffered saline (PBS) (Merk, Germany). Fetal Bovine serum (GIBCO, UK). A plate reader (EXL 800, USA).

*2.3. Extraction and isolation*

The methanol (10 L x 5) was used for extract *Euphorbia tithymaliodes* L. powdered stems (5 kg) at 25ºC. At temperature not above 40ºC, the collective extracts were concentrated under lowered pressure and dried at 25ºC to give 50 g as semi-solid residue. In 100 ml MeOH, the methanolic extract was resuspended, diluted with distilled water to 250 ml, defatted with pet ether and then with $CH_2Cl_2$ was extracted. After evaporation of the solvents, it gave 20 g of pet ether extract and 4.2 g $CH_2Cl_2$ extract, respectively. Apart of pet ether extract (19 g) on silica gel column (1.5 m x 3 cm) was chromatographed and eluted starting with 100 % pet ether and then increasing polarity with EtOAc up to 30 %, collecting 250 ml fractions. By TLC the collected fractions were monitored, by spraying with vanillin/$H_2SO_4$ were visualized and then heating at 120ºC until maximum progress of the color spot. Subfractions eluted with pet ether-EtOAc (90:10) (117.7 mg) contained compound **1** which was cleansed on silica gel column (50 x 1cm) eluted with pet ether-EtOAc (95:5) to give pure compound **1** (20 mg) as colorless needless; $R_f$ = 0.90; $CH_2Cl_2$-MeOH (9.5:0.5). Further subfractions eluted with pet ether-EtOAc (80:20) (50 mg) on silica gel column (30 x 1 cm) rechromatographed to yield compound **2** (3 mg) as colorless needles; $R_f$ = 0.87; $CH_2Cl_2$-MeOH (9.5:0.5). Apart of $CH_2Cl_2$ extract (4.0 gm) on silica gel column (1.0 m x 1.0 cm) was chromatographed and eluted with 100 % $CH_2Cl_2$ and then increasing polarity with MeOH up to 10 %, collecting 250 ml fractions. Subfractions eluted with $CH_2Cl_2$ (100 %) yielded compound **3** (3 mg) as white needle crystals; $R_f$ = 0.19; $CH_2Cl_2$-MeOH (90:10). Further subfractions eluted with $CH_2Cl_2$-MeOH (99:1) (734 mg) on silica gel column (40 cm x 1.0 cm) were rechromatographed which eluted with $CH_2Cl_2$-MeOH (95:5) to give compound **4** (7 mg) as colorless needle crystals; $R_f$ = 0.53; pet ether-EtOAc (80:20). Subfractions eluted with $CH_2Cl_2$-MeOH (90:10) (200 mg) contained compound **5** (3.8 mg) which was purified by prep HPLC column eluted with hexane-EtOAc (70:30) and on silica gel column (60 x 0.5 cm) rechromatographed and then eluted with Hexane- EtOAc (80:20) to give compound **5** (3.8 mg); $R_f$ = 0.19; Hexane-EtOAc (80:20). Other subfractions (200 mg) eluted with $CH_2Cl_2$-MeOH (60:40) were

also purified by prep HPLC column eluted with hexane-EtOAc (70:30) to yield compound **6** (20 mg) as colorless amorphous solid; $R_f$ = 0.16; Hexane-EtOAc (80:20).

**2.3.1. Compound 1** (3-oxo-friedelane): colorless needles, $^1$H and $^{13}$C-NMR (500, 125 MHz, $CDCl_3$): (Tables 1 & 2), ESI-MS: *m/z* = 425 $[M-H]^-$, $C_{30}H_{50}O$, m.p 266-267 ºC. The published m.p (265-267ºC).

**2.3.2. Compound 2** (friedelan-3β-ol): colorless needles, $^1$H and $^{13}$C-NMR (500, 125 MHz, $CDCl_3$): (Tables 1 & 2), ESI-MS: *m/z* = 427 $[M-H]^-$, $C_{30}H_{52}O$, m.p 280-282ºC. The published m.p (281-282ºC).

**2.3.3. Compound 3** (β - sitosterol): white needles, IR (KBr): $\upsilon_{max}$ $cm^{-1}$ 3447 (OH stretching), 1639 (C=C stretching), 1463 ($CH_2$ bending), 1380 ($CH_3$ bending), 1085 (C-O stretching), m.p 136-138ºC. The published m.p (134-136ºC).

**2.3.4. Compound 4** (euphane-7, 24-diene, 3-β-ol; butyrospermol): colorless needle crystals, $^1$H and $^{13}$C-NMR (APT spectrum) (400, 100 MHz, $CDCl_3$): (Tables 1 & 2), ESI-MS: *m/z* = 425 $[M-H]^-$, $C_{30}H_{50}O$, m.p 109-110 ºC. The published m.p (108-110ºC).

**2.3.5. Compound 5** (euphane 7, 25-dienes, 3, 24 β-diol): colorless amorphous solid, $^1$H-NMR and $^{13}$C-NMR (APT spectrum) **(**400, 100 MHz, $CDCl_3$): (Tables 1 & 2), ESI-MS: *m/z* = 441 $[M- H]^-$, $C_{30}H_{50}O_2$.

**2.3.6. Compound 6** (1 α, 13 β, 14 α-trihydroxy-3 β, 7 β-dibenzenzoyloxy-9 β, 15 β-diacetoxyjatropha-5, 11-*E*-diene) : colorless amorphous solid, $^1$H-NMR ($CDCl_3$, δ, ppm, *J* / Hz 400 MHz ): 4.23 (1H, dd, *J* = 3.2, 11.6, H-1), 2.31 (1H, m, H-2), 5.47 (1H, dd, *J* = 4.4, 4, H-3), 4.12 (1H, dd, *J* = 4.8, 10, H-4), 5.80 (1H, d, *J* = 9.6, H-5), 5.29 (1H, m, H-7), 2.08 (2H, m, $H_a$-8 & $H_b$-8), 5.17 (1H, dd, *J* = 3.2, 2.8, H-9), 5.54 (1H, d, *J* = 15.2, H-11), 5.31 (1H, d, *J* = 15.6, H-12), 4.34 (1 H, d, *J* = 4, H-14), 0.98 (3 H, d, *J* = 6.8, $H_3$-16), 1.85 (3 H, s, $H_3$-17), 0.97 (3 H, s, $H_3$-18), 0.99 (3H, s, $H_3$-19), 1.36 (3H, s, $H_3$-20), 1.70 (3H, s, $H_3$-9 ), 2.46 (3H, s, $H_3$-15 ), 7.64 ( 2H, dd, *J* = 7.5, 1.0, H-2`, H-6`), 7.53 (2H, dd, *J* = 7.7, 7.6, H-2``, H-6``), 7.01 (2H, dd, *J* = 7.6, 8.0, H-3`, H-5`), 7.00 ( 2H, dd, *J* = 7.6, 8.0, H-3``, H- 5``), 7.29 (1H, m, H-4`),7.29 (1H , m, H-4``), 4.38 (1H, d, *J* = 3.2, H of OH-1), 2.77 (1H, s, H of OH-13), 4.47 (1H, d, *J* = 3.2, H of OH-14). $^{13}$C-NMR (APT spectrum ) ($CDCl_3$, 100 MHz ): 87.0 (C-1), 43.3 (C-2), 77.6 (C-3), 41.2 (C-4), 119.1(C-5), 134.3 (C-6), 74.3 (C-7), 32.3 (C-8), 74.1 (C-9), 39.7 (C-10), 132.0 (C-11), 129.9 (C-12), 74.7 (C-13), 72.5 (C-14), 91.3 (C-15), 11.7 (C-16), 16.4 (C-17), 23.0 (C-18), 20.6 (C-19), 31.4, 170.2 (C (O -CO-9)), 20.9 ( C ($CH_3$-9)), 173.6 ( C (O-CO-15)), 22.2 ( C ($CH_3$-15)), 165.2 (C (O-CO-3)), 164.9 (C (O-CO-7)), 129.5 (C (1``)), 129.6 (C (1`)), 128.1 (C (3`, 5`)), 127.8 (C (3``, 5``)),132.6 (C-4`), 132.2 (C- 4``), 87.0 (C-1), 74.7 ( C-13), 72.5 (C-14). ESI-MS: *m/z* = 701.3 $[M+ Na]^+$, $C_{38}H_{46}O_{11}$.

**Table 1**

$^1$H-NMR data of compounds 1-2 & 4-5

| $^1$H | 1[a] | 2[b] | 4[a] | 5[b] |
|---|---|---|---|---|
| **1** | 1.67 (2H, m) | 1.73 (2H, dt, *J* = 10, 5) | 1.13 (2H, m) | 1.18 (2H, m) |
| **2** | 2.30 (2H, m) | 1.9 (2H, qd, *J* = 10, 5) | 1.50 (2H, m) | 1.57 (2H, m) |
| **3** | …… | 3.72 (2 H, m) | 3.25 (1H, dd, *J* = 11, 2) | 3.18 (1H, dd, *J* = 4.4, 10.8) |
| **4** | 2.25 (1H, m) | 1.26 (1H, m) | …….. | ……. |
| **5** | …….. | ……. | 1.32 (1H, m) | 1.36 (1H, m) |
| **6** | 1.30 (2H, m) | 1.1 (2H, m) | 1.94 (2H, m) | 1.9 (2H, m) |
| **7** | 1.39 (2H, m) | 1.40 (2H, m) | 5.25 (1H, d, *J* = 2.8) | 5.18 (1H, m) |
| **8** | 1.42 (1H, m) | 1.30 (1H, m) | ……. | …… |
| **9** | ……. | ……. | 2.21 (1H, m) | 2.12 (1H, m) |
| **10** | 1.48 (1H, m) | 0.89 (1H, d, *J* = 10) | …… | …… |
| **11** | 1.26 (2H, m) | 1.43 (2H, m) | 0.9 (2H, m) | 1.2 (2H, m) |
| **12** | 1.33 (2H, m) | 1.35 (2H, m) | 1.80 (2H, m) | 1.58 (2H, m) |
| **13** | ……. | ……. | ……. | …… |
| **14** | ……. | ……. | …….. | …… |
| **15** | 1.32 (2H, m) | 1.54 (2H, m) | 1.46 (2H, m) | 1.59 (2H, m) |
| **16** | 1.39 (2H, m) | 1.46 (2H, m) | 1.26 (2H, m) | 1.2 (2H, m) |
| **17** | …… | ……. | 1.47 (1H, m) | 1.4 (1H, m) |
| **18** | 1.54 (1H, m) | 1.56 (1H, dd, *J* = 10, 5) | 0.80 (3H, s) | 0.74 (3H, s) |
| **19** | 1.22 (2H, m) | 1.38 (2H, m) | 0.74 (3H, s) | 0.67 (3H, s) |

| | | | | |
|---|---|---|---|---|
| **20** | ……. | ……. | 1.37 (1H, m) | 1.4 (1H, m) |
| **21** | 1.30 (2H, m) | 1.48 (2H, m) | 0.80 (3H, d, *J* = 6.8) | 0.79 (3H, d, *J* = 7.6) |
| **22** | 0.95 (2H, m) | 0.82 (2H, d, *J* = 10) | 1.27 (2H, m) | 1.2 (2H, m) |
| **23** | 0.88 (3H, d, *J* = 5.6) | 0.94 (3H, d, *J* = 7.5) | 1.95 (2H, m) | 1.7 (2H, m) |
| **24** | 0.72 (3H, s) | 0.96 (3H, s) | 5.08 (1H, t, *J* = 6.8) | 3.95 (2H, t, *J* = 6.4) |
| **25** | 0.87 (3H, s) | 0.86 (3H, s) | …….. | …… |
| **26** | 1.01 (3H, s) | 0.99 (3H, s) | 1.60 (3H, s) | 4.77 (1H, s), 4.86 (1H, s) |
| **27** | 1.05 (3H, s) | 0.99 (3H, s) | 1.68 (3H, s) | 1.60 (3H, s) |
| **28** | 1.18 (3H, s) | 1.17 (3H, s) | 0.97 (3H, s) | 0.90 (3H, s) |
| **29** | 0.95 (3H, s) | 1.01 (3H, s) | 0.86 (3H, s) | 0.81 (3H, s) |
| **30** | 0.99 (3H, s) | 0.98 (3H, s) | 0.97 (3H, s) | 0.90 (3H, s) |

[a] Spectra run at 500 MHz in $CDCl_3$, δ values in ppm and coupling constants (*J*) in Hz.
[b] Spectra run at 400 MHz in $CDCl_3$, δ values in ppm and coupling constants (*J*) in Hz.

**Table 2**
$^{13}C$-NMR data of compounds 1-2 & 4-5

| **$^{13}C$** | **1[a]** | **2[b]** | **4[a]** | **5[b]** |
|---|---|---|---|---|
| **1** | 22.7 | 37.2 | 15.7 | 37.2 |
| **2** | 41.6 | 27.6 | 36.0 | 27.7 |
| **3** | 213.4 | 79.3 | 72.8 | 79.3 |
| **4** | 58.2 | 39.0 | 49.1 | 38.9 |
| **5** | 42.2 | 50.6 | 37.8 | 50.6 |
| **6** | 41.3 | 24.0 | 41.7 | 23.9 |
| **7** | 18.3 | 117.8 | 17.5 | 117.9 |
| **8** | 53.2 | 145.9 | 53.2 | 145.9 |
| **9** | 37.5 | 48.9 | 37.1 | 48.9 |
| **10** | 59.5 | 34.9 | 61.3 | 35.0 |
| **11** | 35.7 | 18.2 | 35.3 | 18.2 |
| **12** | 30.6 | 33.8 | 30.6 | 33.9 |
| **13** | 39.8 | 43.5 | 38.3 | 43.6 |
| **14** | 38.4 | 51.3 | 39.6 | 51.2 |
| **15** | 32.5 | 34.3 | 32.3 | 33.7 |
| **16** | 36.1 | 28.5 | 35.5 | 28.4 |
| **17** | 30.1 | 53.2 | 30.0 | 53.3 |
| **18** | 42.8 | 18.6 | 42.8 | 22.1 |
| **19** | 35.4 | 22.1 | 35.1 | 13.1 |
| **20** | 28.3 | 35.9 | 28.2 | 36.1 |
| **21** | 32.5 | 18.6 | 32.8 | 18.7 |
| **22** | 39.3 | 35.2 | 39.3 | 30.8 |
| **23** | 6.9 | 25.5 | 11.6 | 32.2 |
| **24** | 14.7 | 125.1 | 16.4 | 76.7 |
| **25** | 18.0 | 130.9 | 18.2 | 147.6 |
| **26** | 20.3 | 17.7 | 20.1 | 111.3 |
| **27** | 18.8 | 25.8 | 18.6 | 17.4 |
| **28** | 32.2 | 27.6 | 32.1 | 27.6 |
| **29** | 35.1 | 27.3 | 35.0 | 14.7 |
| **30** | 31.9 | 14.8 | 31.8 | 27.4 |

[a] $^{13}C$ spectra run at 125 MHz in $CDCl_3$.
[b] APT spectra run at 100 MHz in $CDCl_3$.

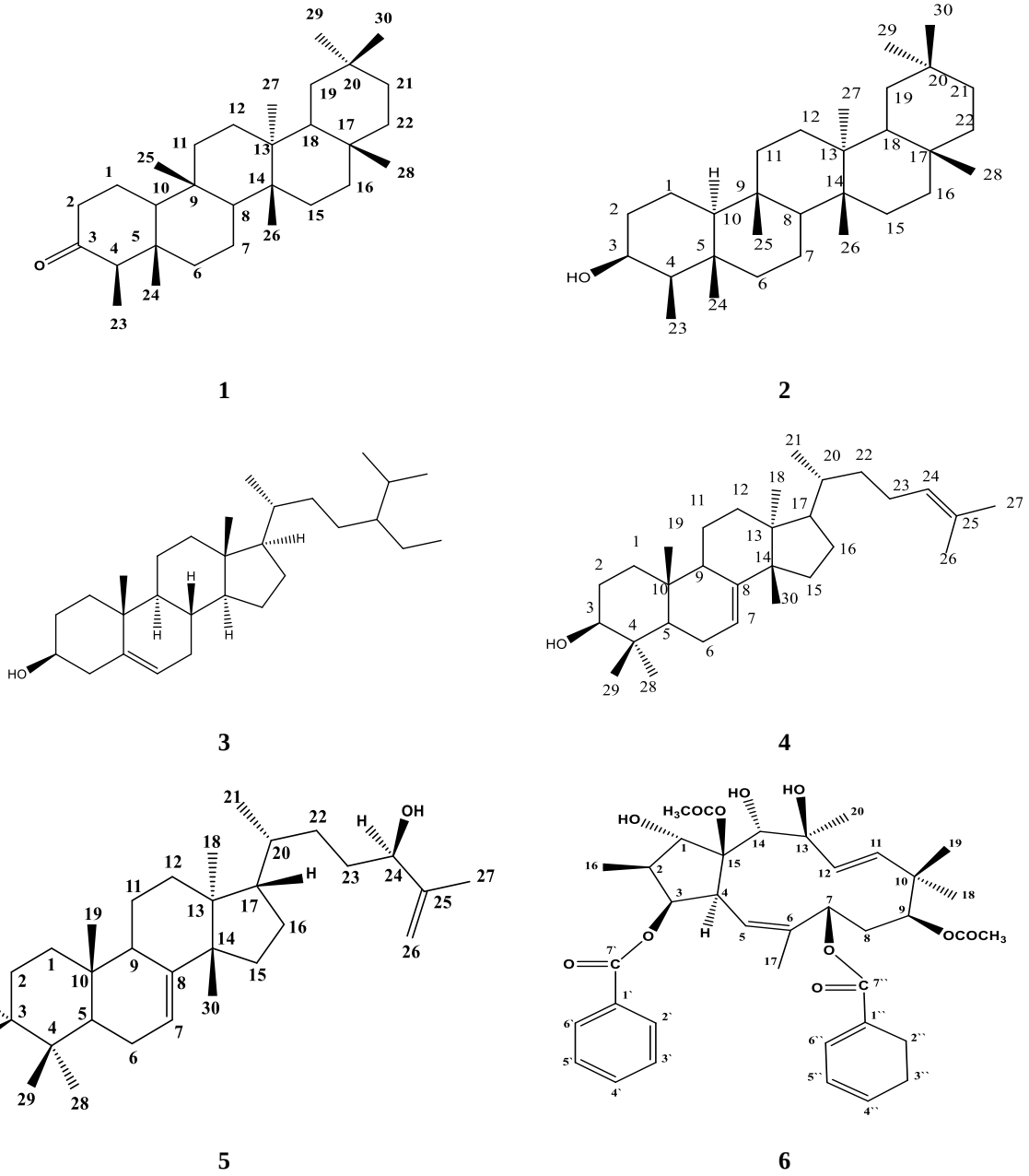

**Figure 1.** Structures of isolated compounds

*2.4. Biological assays*

*2.4.1. Iron ascorbate-stimulated lipid peroxidation in rat liver homogenate*

The assay which was defined by (Baratta *et al.*,1998; Shanmugasundaram and Venkataraman, 2006), was carried out in male albino Sprague Dawley rats liver homogenate. In brief, the reaction mixture consisted of 250 µL liver homogenate (10 % rat liver in KCL 1.1 % w/v), 50 µL of 0.1 mM ascorbic acid, 50 µL of 4 mM ferric chloride,750 µL of TBA (0.8 % w/v) suspended in SDS (0.1 % w/v), 750 µL of acetic acid (20 % v/v) adjusted to PH 3.5 with 1 N NaOH and 50 µL of different concentrations of test substances suspended in SDS (8.1 % w/v). Distilled water was added to a final volume of 2 ml. The mixtures were mixed in a vortex mixer for 5 seconds. At 95°C for 60 minutes, a thermostatic water bath was used for heating the reaction mixtures. Butanol (2.5 µL) was added to each test tube after rapid cooling and then all test tubes were mixed in a vortex mixer for 5 seconds. The mixtures were centrifuged at 1500 x for 3.5 minutes and MDA formed was measured following a modified

thiobarbituric acid reactive species assay as defined by Baratta *et al.,* 1998, against a fully oxidized control. Protection of lipid peroxidation by substrate was calculated as follows:

$$\text{Inhibition of lipid peroxidation} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

*2.4.2. In vivo hepatoprotective assay*

Before beginning the experiment, male albino Sprague Dawley rats (180-200 gm) were permitted free admission to tap water and a standard meal and preserved in a controlled environment at room temperature ± 2°C under 12 hrs., dark/light cycle and adapted at least for 7 days. Rats were separated into 4 groups of 6 each. The control group was treated with a single dose of $CCl_4$ (1 ml/kg, i.p., suspended in corn oil). The normal group received carboxy methyl cellulose 2 % w/v orally (2 ml/kg) daily per one week. The treated group was administered $CCl_4$ (1 ml/kg, i.p., suspended in corn oil) and then treated with pet ether extract every other day for one week (200 mg/kg, orally, suspended in CMC 2 % w/v). The positive control group taken silymarin (50 mg/kg, orally, dissolved in CMC 2 % w/v) every other day for one week. At the end of the experiment animals were anesthetized by diethyl ether 24 h after the last treatment and puncture of the retro-orbital plexus was used for collecting the blood to determine liver enzymes. The cervical dislocation method was used for killed animals and livers were separated, quickly frozen and saved for liver peroxidation assay. A spectrophotometric diagnostic kit according to the manufacturer's directions was used for measuring serum ALT, AST and glutathione. The liver peroxidation was assessed by determination of the formation of the thiobarbituric acid reactive species malondialdehyde (MDA) in rat liver homogenate by a colorimetric method (Baratta *et al.*, 1998).

*2.4.3. Measurement of potential cytotoxic activity of compound 6*

Four human cell lines *viz.*; HepG2 (hepatocellular carcinoma), MCF-7 (breast adenocarcinoma), PC-3 (human prostate cancer) and HCT-116 (colon cancer) were used to determine the inhibitory effects of compound **6** on cell growth using MTT assay (Minh *et al.*, 2014). Cells were plated at 37°C for 24 hours on 96-well plates in RPMI-1640 medium at a density of $10^4$ cells/well in a humidified atmosphere (5 % $CO_2$) (Han *et al.*, 2011). Different concentrations of the test compound (100, 50, 25, 12.5, 6.25, 3.125, and 1.56 µM) were added to each well and cocultured for 48 hours. PBS was used for washing the treated cells and 100 µl of MTT solution (5 mg/ml MTT stock in PBS diluted to 1 mg/ml with 10 % RPMI-1640 medium) were added to each well and incubated at 37°C for four hours. At the end of the assay, 100 µL of DMSO were added and by using a plate reader, optical densities at 570 nm were measured. Doxorubicin was used as positive control.

The viability of the cells treated with test compound was identified as:

$$\text{\% Cell viability} = \frac{\text{average OD of treated samples}}{\text{average OD of untreated samples}} \times 100$$

*2.4.4. Statistical analysis*

Variances among all groups were examined by one-way analysis of varience (ANOVA) followed by Tukey–Kramer multiple comparisons tests for assessment of response variation among different groups N = 6. The results are expressed as mean values ± SE from 3 isolate experiments. Differences between means were considered significant at $p<0.05$. GraphPad Prism 7.0 software was used for performing the analyses (GraphPad Software, Inc., San Diego, CA, USA).

**Approval for animal experiments**

This research work conducted on animals has been approved by the Research Ethics Committee, code no. [2022-44]; Faculty of pharmacy-Mansoura university.

## 3. Results and Discussion

*3.1. Identification of the compounds*

MeOH was used for extraction the stems of *Euphorbia tithymaloides* L. and then the extract was fractionated between $H_2O$ and organic solvents of increasing polarities *viz.*; pet ether and $CH_2Cl_2$. Column chromatography of pet ether extract on silica gel gave compounds **1** & **2** while that of $CH_2Cl_2$ extract gave compounds **3**-**6**. The isolated compounds were recognized by comparing their physical and spectral data; $^1H$ & $^{13}C$-NMR, APT, HMBC, $^1H$–$^1H$ NOESY and ESI mass spectrometry to published ones.

**Compound 1** was isolated as colorless needles (20 mg) from pet ether extract. A pseudo molecular ion peak in ESI-MS at m/z 425 $[M-H]^-$, indicated a molecular formula of $C_{30}H_{50}O$. $^1H$-NMR data (Table 1) indicated the presence of seven tertiary methyl groups and a secondary methyl group at δ 0.88 appearing as doublet $J$ = 5.6 Hz. $^{13}C$-NMR spectrum (Table 2) showed the presence of 30 carbon resonances suggesting a triterpene derivative. These were discriminated into 8 methyls at δ 6.9, 14.7, 18.0, 20.3, 18.8, 32.2, 35.1 and 31.9, 11 methylenes at δ

22.7, 41.6, 41.3, 18.3, 35.7, 30.6, 32.5, 36.1, 35.4, 32.5 and 39.3, 4 methines at δ 58.2, 53.2, 59.5 and 35.5 and 7 quaternary carbons including a keto carbonyl at δ 42.2, 37.5, 39.8, 38.4, 30.1, 28.3 and 213.4, respectively. Since the molecular formula indicated six degrees of unsaturation, this compound was shown to be a pentacyclic triterpene with a keto function. The presence of signals due to 1 secondary methyl at δ 28.3 and 7 tertiary methyls at δ 58.2, 42.2, 37.5, 39.5, 38.4 and 30.1 in the $^{13}$C-NMR data suggested a friedlane skeleton. These data were comparable to those published by De Andrade, *et al.,* 2008 which confirmed compound **1** to be friedelin or 3-oxo-friedelane (Figure 1). This compound is isolated from *Euphorbia tithymaliodes* for the first time.

**Compound 2** was isolated as White needles (3 mg) from pet ether extract. A pseudo molecular ion peak in ESI-MS at m/z 427 [M-H] $^-$, indicated a molecular formula of $C_{30}H_{52}O$. $^1$H-NMR data (Table 1) showed 7 singlet methyl groups at δ 0.86, 0.95, 0.97, 0.99, 1.01 and 1.17 in addition to a secondary methyl group at δ 0. 94 (3 H, d, *J* = 7.5). $^{13}$C-NMR data (Table 2) showed 30 carbon resonances including 7 tertiary methyls at δ 16.4, 18.2, 18.6, 20.1, 31.8, 32.1 and 35.0; a secondary methyl group at δ 11.6; 11 methylenes at δ 15.7, 17.5, 30.6, 32.3, 32.8, 35.0, 35.3, 35.5, 36.0, 39.3, and 41.7; 5 methines at δ 42.8, 49.1, 53.2, 61.3, 72.8 and 6 quaternary carbons at δ 28.2, 30.0, 37.1, 37.8, 38.3, and 39.6. These collective data suggested a friedlane skeleton. A hydroxy methine proton at δ 3.72 (2H, m) indicated a hydroxyl group at C-3. The NMR data of compounds **1** & **2** were very similar except for the presence of an oxygenated secondary methine carbon at δ 72.8 in **2** instead of the keto function at δ 213.4 in **1** so the structure of compound **2** was determined to be friedelan-3β-ol. Based on the spectral data (Tables 1, 2) and by comparison with those reported for friedelan-3β-ol (Minh *et al.,* 2014) the structure of compound **2** was definite as friedelan-3β-ol (Figure 1). It was previously separated from the fresh stems and leaves of *E. tithymaloides* (Minh *et al.*, 2014).

**Compound 3** was isolated as white needles (3 mg) from $CH_2Cl_2$ extract; It was identified as β-sitosterol (Figure 1) based on comparing its IR spectrum & melting point to published data (Minh *et al.*, 2014). It was previously separated from the fresh leaves and stems of *E. tithymaliodes* (Minh *et al.*, 2014).

**Compound 4** was isolated as colorless needle crystals (7 mg) from $CH_2Cl_2$ extract. A pseudo molecular ion peak in ESI-MS at m/z 425 [M-H] $^-$, indicated a molecular formula of $C_{30}H_{50}O$ with six degrees of unsaturation. The $^1$H-NMR data (Table 1) indicated the presence of five tertiary methyls at δ 0.74, 0.80, 0.86, and 0.97 (6 H, s); a secondary methyl at δ 0.80 (3H, d, *J* = 6.8*)*, two vinyl methyl singlets at δ 1.60 and 1.68. $^{13}$C-NMR data (Table 2) showed 30 carbon resonances separated by APT experiment into eight methyl groups at δ 18.6, 22.1, 18.6, 17.7, 25.8, 27.6, 27.3, 14.8; nine methylenes at δ 37.2, 27.6, 24.0, 18.2, 33.8, 34.3, 28.5, 35.2, 25.5; seven methines at δ 35.9, 48.9, 50.6, 53.2, 79.3, 117.8, 125.1 including an oxygen bearing carbon at δ 79.3 and six quaternary carbons at δ 34.9, 39.0, 43.5, 51.3, 130.9, 145.9. From these data suggested that compound **4** would be a derivative of tetracyclic triterpene of euphane-type. Correlation peaks in HMBC spectrum (Figure 2) between H-30 (δ 0.97, 3 H, s) and C-8 (δ 145.9); H-7 (δ 5.25, 1H, d, *J* = 2.8 Hz) and C-9 (δ 48.49) confirmed a double bond between C-7 and C-8 (δ 117.8, 145.9) respectively. A hydroxymethine proton at δ 3.25 (1 H, dd, *J* = 11, 2) which was assigned to C-3 by cross peak in HMBC spectrum between H-3 (δ 3.25,1 H, dd, *J* = 11.2) and C-29 (δ 14.75) & C-28 (δ 27.61). HMBC correlations between H-24 (δ 5.08, 1H, t, *J* = 6.8) and C-26 (δ 17.7); H-26 (δ 1.60, 3 H, s) and C-24 (δ 125.1) & C-25 (δ 130.9) confirmed a double bound between C-24 and C-25. These data were comparable with those published for butyrospermol (Wu *et al.*, 2006) and confirmed compound **2** to be euphane-7, 24-diene 3-β-ol (butyrospermol) (Figure 1). This is the first report for its isolation from *Euphorbia tithymaliodes*.

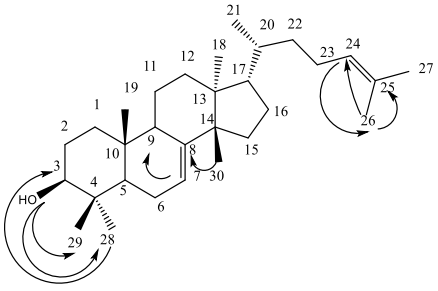


**Figure 2**. Selected HMBC correlations of compound **4**

**Compound 5** was isolated as colorless amorphous solid (3.8 mg) from $CH_2Cl_2$ extract. A pseudo molecular ion peak in ESI-MS at m/z 441 [M-H] $^-$, indicated a molecular formula of $C_{30}H_{50}O_2$ with six degrees of unsaturation. The $^1$H-NMR spectrum (Table 1) exhibited signals assignable to 6 tertiary methyls at δ 0.74, 0.67, 1.60, 0.90, 0.81

and 0.90; a secondary methyl at δ 0.79 (3H, d, *J* = 7.6) and one vinyl methyl at δ 1.60 (3H, s). The $^{13}$C-NMR data (Table 2) showed 30 carbon resonances, discriminated by APT experiment into 7 methyl groups at δ 22.1, 13.1, 18.7, 17.4, 27.6, 14.7, 27.4 in addition to 11 methylene carbons at δ 37.2, 27.7, 32.9, 18.2, 33.9, 33.7, 28.4, 30.8, 32.2, 76.7, 111.3, 6 methines at δ 79.3, 50.6, 117.9, 48.9, 53.3, 36.1 and 6 quaternary carbons at δ 38.9, 145.9, 35.0, 43.6, 51.2, 147.6. The collective spectral data of compound **5** suggested its structure to have euphane type triterpenoid skeleton. The $^{1}$H-NMR and $^{13}$C-NMR (Tables 1 & 2) suggested the presence of two substituted double bounds at C-7 (δ 117.9) and C-25 (δ 147.6). Selected HMBC correlations (Figure 3) between $H_3$-28 (δ 0.90, 3H, s), $H_3$-29 (δ 0.81, 3H, s) and carbon resonance at (δ 79.3) confirmed that an oxygen bearing carbon was present at C-3. Correlations peaks in HMBC between H-26 (δ 4.86, 1H, s), H-27 (δ 1.60, 3H, s) and carbon resonance at (δ 76.7) suggested that a hydroxy group was located at C-24. Large chemical shift at H-3 (1H, 3.18, dd, *J* = 4.4, 10.8) and at H-24 (1H, 3.95, t, *J* = 6.4) which indicated that the two hydroxyl groups at C-3 at δ 79.3 and C-24 at δ 76.7. Correlations peaks in HMBC between H-27 (δ 1.60, 3H, s) and the quaternary carbon resonance at (δ 147.6) and the olefinic carbon resonance at (δ 111.3) confirmed a double bound at C-25. Correlation peaks in HMBC between H-30 (δ 0.90, 3 H, s) and C-8 (δ 145.9). Moreover, correlation peaks in NOESY experiment (Figure 4) between $H_3$-21 (δ 0.79, 3H, d, *J* = 7.6) and $H_3$-18 (δ 0.74, 3H, s) supported α orientation of C-18 & C-21, while correlation peaks between $H_3$-29 (δ 0.81, 3H, s) and $H_3$-19 (δ 0.67, 3H, s) supported the β orientation of both $_{CH3}$ groups. Correlations peaks in NOESY between H-3 α (δ 3.18, 1H, dd, *J* = 4.4, 10.8) / H-1 α (δ 1.18, 2H, m), H-2 α (δ 1.57, 2H, m), H-5 α (δ1.36, 1H, m), $H_3$-28 α (δ 0.90, 3H, s) supported the α orientation at these positions. NOESY correlations are cited in the (Figure 4). These data were comparable to those reported for euphane-7, 25 diene 3, 24-diols (β) (Zong *et al.*, 2011), so the identity of compound **5** was confirmed as euphane-7, 25 diene 3, 24-diols (β) (Figure 1) which is reported for the first time from *Euphorbia tithymaliodes*.

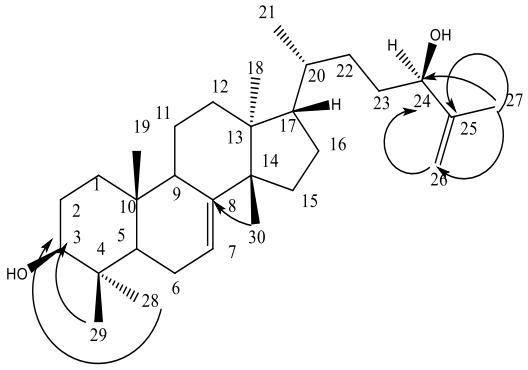


**Figure 3**. Selected HMBC correlations of **5**

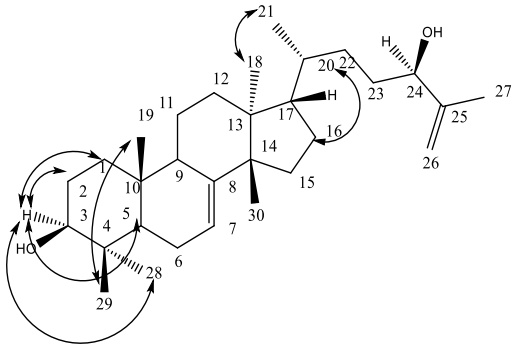


**Figure 4.** Selected NOEY correlations of **5**

**Compound 6:** was isolated as colorless amorphous solid (20 mg) from $CH_2CL_2$ extract. A pseudo molecular ion peak in ESI-MS at 701.3 [M+ Na] $^{+}$ indicated a molecular formula of $C_{38}H_{46}O_{11}$. The molecular formula is corresponding to ten degrees of unsaturation. The $^{1}$H-NMR spectrum showed two benzyl groups at δ 7.64 (2H, dd, *J* = 7.5, 1.0, H-2`, H-6`), 7.29 (1H, m, H 4`) and 7.01 (2H, dd, *J* = 7.6, 8.0, H-3`, H-5`) and at δ 7.53 (2H, dd, *J* = 7.7, 7.6, H-2``, H-6``), 7.29 (1H, m, H-4``) and 7.00 (2H, dd, *J* = 7.6, 8.0, H-3``, H-5``); three olefinic protons at δ 5.80 (1H, d, *J* = 9.6, H-5), 5.54 (1H, d, *J* = 15.2, H-11) and 5.31 (1H, d, *J* = 15.6, H-12); two hydroxy methine protons at 4.23 (1H, dd, *J* = 3.2, 11.6, H-1) and 4.34 (1H, d, *J* = 4, H-14). In addition to five methyl group at δ 0.98 (3H, d, *J* = 6.8, $H_3$-16), 1.85 (3H, s, $H_3$-17), 0.97 (3H, s, $H_3$-18), 0.99 (3H, s, $H_3$-19) and 1.36 (3H, s, $H_3$-20). The $^{13}$C-NMR (APT spectrum) showed 20 carbon resonances crossponding to five methyl groups, one methylene, ten methines and four quaternary carbons (Figure 1). $^{13}$C-NMR carbon showed signals attributable two benzyl groups at δ 77.6 & 74.3; two acetyl groups at δ 74.9 & 22.2; two double bound pairs at δ 119.1 & 132.0. These data indicated that the structure was belonging to jatrophane diterpene nucleus. A highly oxygenated *trans*-bicyclo [10.3.0] pentadecane framework is characteristic for jatrophane diterpenes where in there were 5 methyls separately located at C-2, C-6, C-10 x 2 and C-13 (Rinner, 2015). It was well-known from the occurrence of 5 methyl groups, which one was secondary methyl and two of which were tertiary methyls bonding to the same carbon ($CH_3$-C-$CH_3$) as detected from HMBC (Figure 5) correlations of $H_3$-18 (δ 0.97, 3H, s) / C-19 (δ 20.6) and C-11 (δ 132.0); $H_3$-19 (δ 0.99, 3H, s) / C-18 (δ 23.0) and C-12 (δ 129.9). Attachment of benzyloxy groups at C-3 (δ 77.6) and C-7 (δ 74.3), and acetoxy group at C-9 (δ 74.1) were determined from correlations peaks in HMBC between H-3 (δ 5.47, 1 H, dd, *J* = 4.4, 4), H-2` (δ 7.64, 1H, dd, *J* = 7.5, 1.0) and H-6` (δ 7.64, 1H, dd, *J* = 7.5, 1.0) / OCO-3 (δ 165.2); H-7 (δ 5.29, 1H, m), H-2`` (δ 7.53, 1H, dd, *J* = 7.7, 7.6) and H-6`` (δ 7.53, 1H, dd , *J* = 7.7, 7.6) / OCO-7 (δ 164.9);

$CH_3COO$-9 (δ1.70, 3H, s) and H-9 (δ 5.17, 1H, dd, *J* = 3.2, 2.8) ) / OCO-9 (δ 170.2), respectively. The placement of double bounds at C-5 (δ 119.1) and C-11 (δ 132.0) was established by correlations peaks in HMBC between H-5 (δ 5.80,1 H, d, *J* = 9.6) / C-6 (δ 134.3), C-15 (δ 91.3), C-17 (δ 16.4) and between H-12 (δ 5.31, 1H, d, *J* = 15.6) / C-10 (δ 39.7) and C-20 (δ 31.4). The geometry of $\Delta^{11}$ double bond was assigned as *trans* depending on the large coupling constant between H-11 and H-12 (*J* = 15.6). The relative configuration of compound **6** was determined through a NOESY experiment (Figure 6) and by comparison of the coupling constants pattern assuming a *trans*-ring junction of the cyclopentane ring and an α orientation for H-4, characteristic to jatrophane diterpenes (Vasas *et al*., 2011). The NEOSY correlations between H-4 (δ 4.12, 1H, dd, *J* = 4.8, 10.0) / H-2 (δ 2.31, 1 H, m), H-3 (δ 5.47, 1H, dd, *J* = 4.4, 4.0) and $H_3$-17 (δ 1.85, 1H, s) supported the α orientation of these protons also supported the β orientation of methyl group at C-2 and benzyloxy group at C-3. Correlation peaks in NOESY spectrum between OH-14 (δ 4.47, 2 H, d, *J* = 3.2) / H-2 (δ 2.31, 1H, m) supported the α orientation of hydroxyl group at C-14. Cross peaks between OH-13 (δ 2.77, 1H, s) / H-14 (δ 4.47,1H, d, *J* = 3.2) supported α orientation of methyl group at C-13. Correlation peaks between H-11 (δ 5.54,1H, d, *J* = 15.2) & H-4 (δ 4.12, 1H, dd, *J* = 4.8, 10.0) & H-11 (δ 5.54,1H, d, *J* = 15.2) / $H_3$-18 (δ 0.97, 3H, s) supported α orientation of $CH_3$ group. Correlation peaks between H-9 (δ 5.17,1H, dd, *J* = 3.2, 2.8) / $H_3$-18 (δ 0.97,3H, s) supported α orientation of $CH_3$ and β orientation of acetoxy group at C-9. Correlation peaks between H-9 (δ 5.17,1H, dd, *J* = 3.2, 2.8) / H-8 α (δ 2.08,1H, m) supported the α orientation of this proton at C-8. Correlation peaks between H-8 (δ 2.08,1H, m) / H-7 (δ 5.29,1H, m) supported the α orientation of this proton and β orientation of benzyloxy group at C-7. The previous data confirmed compound **6** to be (1 α, 13 β, 14 α-trihydroxy-3 β, 7 β-dibenzenzoyloxy-9 β, 15 β-diacetoxyjatropha-5, 11-*E*-diene) (Figure 1). This compound was previously isolated from the latex of *Euphorbia tithymaliodes* (Mongkolvisut and Sutthivaiyakit, 2007).

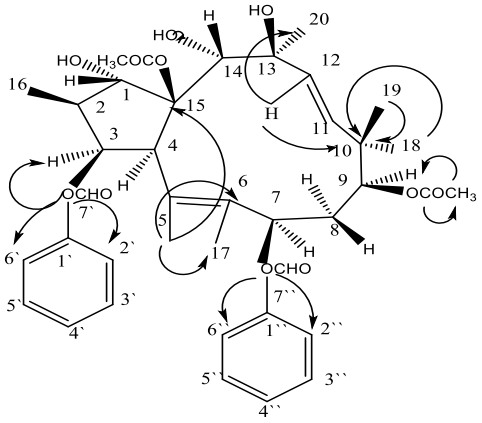

**Figure 5**. Selected HMBC correlations of compound **6**

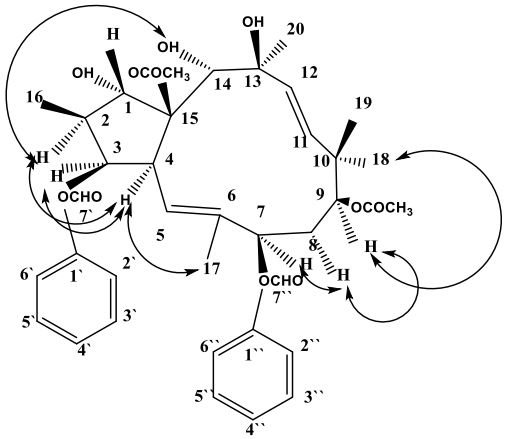

**Figure 6**. Selected NOEY correlations of compound **6**

*3.2. Biological assay*

*3.2.1. Iron ascorbate-stimulated lipid peroxidation in rat liver homogenate*

Pet ether, $CH_2Cl_2$ and MeOH extracts were assessed separately for inhibition of rat liver oxidative stress *in vitro.* Iron-ascorbate system was used for fasting lipid peroxidation in rat liver homogenate. *In vitro* in a nonenzymatic way, liver peroxidation can progress (Fukuzawa *et al.*, 1993; Bondent *et al.*, 2000). Membrane injury of liver cells could affect in the release of oxygen-derived free radicals and other reactive oxygen species (ROS) resulting from lipid peroxidation progressions, that characterizes a general feature of constant inflammatory response and liver injury conducting to necrosis and fibrosis (Shimizu *et al.*, 2005). The pet ether, $CH_2Cl_2$, MeOH extracts and silymarin; as standardized extract from milk thistle which is broadly used as hepatoprotective natural product; were assessed in the similar system. Results of the assay showed that the hepatoprotective activity of the tested extracts from *Euphorbia tithymaliodes* stems was dose-dependent. The pet ether extract displayed the highest hepatoprotective activity at concentrations of 5 and 10 mg/ml, respectively (Table 3), followed by $CH_2Cl_2$ & then MeOH extracts. The hepatoprotective effect of pet ether extract was not significantly dissimilar from that of silymarin generally used as hepatoprotective natural drug from milk thistle fruits of *Silybum marianum* (Wianowsk and Wisniewski, 2014). The results of this assay will lead to consideration of the different extracts from the stems of *Euphorbia tithymaliodes* as a source for hepatoprotective compounds.

**Table 3.**
Results of inhibitory effect of different extracts of the stems of *Euphorbia tithymaliodes* (L.) and silymarin on iron ascorbate-induced lipid peroxidation:

| Concentration mg/ml | % inhibition * | | | |
|---|---|---|---|---|
| | Petroleum ether | Methylene chloride | Methanolic | Silymarin |
| 1 | 34.8 ± 1.9 | 32.2 ± 1.8 | 30.9 ± 1.9 | 32.4 ± 1.3 |
| 5 | 66.6 ± 5.9 | 47.5 ±0.99 | 45.3 ± 0.96 | 44.4 ± 0.98 |
| 10 | 79.2 ± 2.8 | 54.7 ± 1.6 | 52.2 ± 1.9 | 77.8 ± 1.5 |

* Data are expressed as means ± SEM of % inhibition compared to a fully peroxidized control.

*3.2.2. In vivo hepatoprotective activity*

The hepatoprotective activity of Pet ether extract from *Euphorbia tithymaliodes* stems was screened *in vivo* on male albino Sprague Dawely rats which showed the highest hepatoprotective activity *in vitro*. $CCl_4$, a well-known standard compound for the initiation of chemical liverwort damage was used (Bouhrim *et al.*, 2018). By injection of $CCl_4$ to rats, liver transaminases AST and ALT levels were significantly increased. Also, the level of the liver injury marker; MDA; was increased over normal group (Table 4). AST and ALT levels were raised in rats with the $CCl_4$ treatment alone in comparison with the normal control group. This raise in liverwort injury markers has been accredited to cell injury or cell membranes became permeable and their release into the circulation (Kharchoufa *et al.*, 2020). Malondialdehyde (MDA) is used as a marker of oxidative liver damage which is a major product of lipid peroxidation (Bondet *et al.*,2000), decrease of the level of glutathione (GSH) is also an indicator of liver damage. The treated group with pet ether extract showed decreased levels of ALT, AST and MDA compared to $CCl_4$-treated group (Table 4), while GSH showed increased levels nearly to normal values. The protective effect of pet ether extract from *Euphorbia tithymaliodes* stems on liver damage was not significantly different from that of silymarin (milk thistle extract), commonly used as hepatoprotective natural product (Table 4). This is the first report for the hepaprotective activity of pet ether extract of the stems of *Euphorbia tithymaliodes*.

**Table 4.**
Effects of pretreatment with petroleum ether extract on $CCL_4$ induced hepatotoxicity in rats *:

| Markers | Normal | $CCL_4$ | Petroleum ether extract | Silymarin |
|---|---|---|---|---|
| ALT [U/L] | 25.6 ±2.9 | 60.6 ± 4.9 | 44.12 ± 2.8 | 40.48 ± 3.3 |
| AST [U/L] | 111.7 ± 9.3 | 219.2 ± 18.4 | 171.8 ± 12.7 | 138.1 ± 13.7 |
| GSH [mg/dL] | 1.775 ± 0.05 | 0.86 ± 0.07 | 1.29 ± 0.08 | 1.45 ± 0.05 |
| MDA [nmol/g liver] | 2.57 ± 0.07 | 4.57 ± 0.23 | 3.19 ± 0.12 | 2.99 ± 0.09 |

* Data are expressed as means ± SEM.

*3.2.3Measurement of potential cytotoxic activity of compound 6*

Compound **6** was assessed for cytotoxicity against four human tumor cell lines, *viz.*, HepG2 (hepatocellular carcinoma), MCF-7 (breast adenocarcinoma), PC-3 (human prostate cancer) and HCT-116 (colon cancer). Screening of the cytotoxic activity was achieved by using MTT assay (Mosmann, 1983). The $IC_{50}$ values of HepG2, MCF-7, PC-3 and HCT-116 are shown in (Table 5). Doxorubicin (Tacar *et al.*, 2013); (Mohamed *et al.*, 2022) was used as a positive control drug in this assay. Compound **6** isolated from the $CH_2Cl_2$ extract of the stem was active against all tested cell lines. Results of the assay reveal that, compound **6** has the strongest cytotoxicity against HepG2 and HCT-116 cells with $IC_{50}$ values of 12.99 ± 0.9 and 18.63 ± 1.4 μM, respectively but had moderate cytotoxicity against PC-3 and MCF-7 with $IC_{50}$ values of 24.40 ± 1.9 and 37.12 ± 2.3 μM, respectively (Table 5).

**Table 5.**
Results of cytotoxicity screening of compound 6 separated from *Euphorbia tithymaliodes* stems:

| | $IC_{50}$ (μM) * | | | |
|---|---|---|---|---|
| **Compounds** | **HepG2** | **HCT-116** | **MCF-7** | **PC-3** |
| **6** | 12.99 ± 0.9 | 18.63 ± 1.4 | 24.40 ± 1.9 | 37.12 ± 2.3 |
| Doxorubicin** | 4.50 ± 0.2 | 5.23 ± 0.3 | 4.17 ± 0.2 | 8.87 ± 0.6 |

* $IC_{50}$ (μM): 1–10 (very strong), 11–20 (strong), 21–50 (moderate), 51–100 (weak) and above 100 (non-cytotoxic). According to the National Cancer Institute guidelines (Boik, 2001).
** Doxorubicin as a positive control.

## 4- Conclusion

The present study resulted in isolation of six compounds from the stems of *Euphorbia tithymaliodes*. Pet ether extract was found to have the highest hepatoprotective activity *in vitro*. The compounds separated from pet ether extract may have antioxidant activity and thus inhibit the oxidative stress-induced liver injury. The diterpene 1 α, 13 β, 14 α-trihydroxy-3 β, 7 β-dibenzenzoyloxy-9 β, 15 β-diacetoxyjatropha-5, 11-*E*-diene showed strong cytotoxicity against HepG2 and HCT-116 and moderate cytotoxicity against PC-3 and MCF-7.

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