Phytopharmacology

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Chemical constituents, antimicrobial, analgesic, antipyretic, and anti-inflammatory activities of *Euphorbia peplus* L.

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Received: 9 August 2012, **Revised**: 28 September 2012, **Accepted**: 29 September 2012

Abstract

Fifteen compounds were isolated from *Euphorbia peplus* L.. Their structures were established by physical, chemical, and spectral data (UV, IR, MS, and 1D NMR), as well as comparison with authentic samples. The preliminary phytochemical screening of the alcoholic extract was done. GC-MS study of the fatty acid methyl esters of the *n*-hexane fraction was carried out. The antimicrobial, pharmacological, and cytotoxic activities of the different extracts were evaluated. The anti-inflammatory activity was evaluated by using yeast-induced paw edema method at doses of 200 and 400 mg/kg of the extracts. The MeOH and EtOAc extracts give potent anti-inflammatory activity compared with indomethacin. All the extracts exhibited significant analgesic activity in the acetic acid-induced writhing method at dose 400 mg/kg. The tested extracts showed antipyretic activity at doses 200 and 400 mg/kg for each extract. They control the hyperthermia for 4 hr without decrease in activity.

Keywords: *Euphorbia peplus*, Euphorbiaceae, GC-MS, antimicrobial,

Introduction

Family Euphorbiaceae comprises over 7500 species within 283 genera (Boulos, 1980; Tackholm, 1974). The genus *Euphorbia* consists of more than 1600 species growing in nearly all types of climates throughout the world (Boulos, 1980). The wide spread plants of the genus *Euphorbia* are rich source of sterols, flavonoids, diterpenoids, and triterpenes with diverse structures (Jassbi, 2006; Ferreira et al, 1993; Gotta et al, 1984; Ivanovaa et al, 2003; Rizk et al, 1980). *Euphorbia peplus* L. is originally native to Europe and North Africa (Zhi-Qin et al, 2010). Its milky sap has long been used as a remedy for the treatment of skin cancers especially non-melanoma skin cancer (NMSC) and the active compounds have been determined to be diterpene esters (Ramsay et al, 2011). These esters were cytotoxic against a variety of cancers both *in vivo* and *in vitro* and are the subject of ongoing research around the world. Previous phytochemical studies of *E. peplus* led to the isolation of diterpenes (Zhi-Qin et al, 2010; Homanna et al, 1999; Jakopovic et al, 1998; Mucsi et al, 2001), flavonoids (Jas-

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sbi, 2006; Dumkow et al, 1973; Mayumi et al, 1975; Nazemiyeh et al, 2010), sterols (Jassbi, 2006; Ferreira et al, 1993; Giner et al, 2000), triterpene alcohols (Cateni et al, 2000), and cerebrosides (Cateni et al, 2010). In this work we reported the phytochemical screening, isolateion and structural elucidation of fifteen compounds; eight of them (**1**, **4**, **5**, **7**, **10**, and **13-15**) were reported for the first time from the plant and one (**6**) for the first time from the family. Also, GC analysis of the fatty acid methyl esters of the *n*-hexane fraction, in addition to antimicrobial, cytotoxic, and pharmacological activities of the different extracts were carried out.

Materials and methods

General experimental procedures

Melting points were carried out on an Electrothermal 9100 Digital Melting Point apparatus (Electrothermal Engineering Ltd, Essex, England). EIMS was recorded on a Jeol the mass route JMS.600H mass spectrometer. UV spectra were recorded on a Shimadzu 1601 UV/VIS spectrophotometer in MeOH and after addition of different shift reagents. The IR spectra were measured on a Shimadzu Infrared-400 spectrophotometer (Kyoto, Japan). NMR spectra (chemical shifts in ppm, coupling constants in Hz) were recorded on Jeol Oxford NMR YH-400 and 500 using $CDCl_3$ and DMSO-d_6 as solvents. NMR spectra were referenced to the solvent signals ($CDCl_3$: 7.26 ppm for ^{1}H and 77.0 ppm for ^{13}C; DMSO-d_6: 2.49 ppm for ^{1}H and 39.9 ppm for ^{13}C). Column chromatographic separation was performed on silica gel 60 (0.04-0.063 mm, Merck) and Sephadex LH-20 (0.25-0.1 mm, Merck). TLC was performed on pre-coated TLC plates with silica gel 60 F_{254} (0.2 mm, Merck). The chromatograms were developed using the following solvent systems: *n*-hexane:EtOAc (95:5, S_1), *n*-hexane:EtOAc (90:10, S_2), $CHCl_3$:MeOH (95:5, S_3), $CHCl_3$:MeOH (90:10, S_4), $CHCl_3$: MeOH (80:20, S_5), and *n*-BuOH:HOAc:H_2O (4:1:2, S_6). Spots were detected by spraying with the following spray reagents: I-1 % $AlCl_3$ for flavonoids, II-$FeCl_3$ for phenolic compounds, III-*p*- anisaldehyde/H_2SO_4 for triterpenoids (Mohammad et al, 2010).

Plant material

The plant material used in this work was the whole plant of *Euphorbia peplus* L. The plant was collected during the flowering stage in the period of April to June 2006 from Assuit University campus. The plant was kindly identified by Prof. Dr. A. A. Fayed, Professor of Plant Taxonomy, Botany Department, Faculty of Science, Assiut University. A voucher sample (EP-20061) was kept in the herbarium of the Faculty of Pharmacy, Assiut University, Assiut, Egypt.

Phytochemical screening

The alcoholic extract of *Euphorbia peplus* L. was subjected to phytochemical tests to detect the various types of chemical constituents present using standard procedures (Rakesh et al., 2010).

GC-MS analysis

5 g of the *n*-hexane fraction refluxed with 0.5 N alc. KOH for 3 hr on a boiling water bath. The alcohol was distilled off and the aqueous liquid was diluted with distilled water

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then extracted with ether till exhaustion to give unsaponifiable matter: The aqueous solution (soap) that remained after removal of the unsaponifiable matter was treated in the same way as previously mentioned to afford the fatty acids methyl esters (Assaf et al, 2010). GC-MS analysis of the fatty acids methyl esters was performed using Agilent GC-MS spectrometer (USA). The software controller/integrator was Turbo Mass, version 4.5.0.007 (PerkinElmer). GC-MS capillary column (3% methyl phenyl silicon type of stationary phase (OV-17) on 80/100, Carbowax HP (CWHP), 6' x 1/8" x 0.085", S.S.) was used. The carrier gas was helium (purity 99.9999%) at a flow rate of 2 mL/min (32 p.s.i., flow initial 55.8 cm/s, split; 1:40). The column temperature program was: 160 °C for 2 min then increase by rate 15 °C/min till 300 °C and isothermal for 15 min. The injector temperature was 250 °C. Detector temperature is 320 °C with dual flame ionization detector. MS scan was from 50 to 650 *m/z*.

Extraction and isolation

The air-dried powdered plant (2.7 kg) was extracted with MeOH. The MeOH extract was concentrated under reduced pressure to get a viscous brown residue (110 g). This residue was suspended in 500 mL distilled water and subjected to solvent fractionation using *n*-hexane, $CHCl_3$, EtOAc, and *n*-BuOH which were separately concentrated to yield 20, 12, 17, and 12 g, respectively.

Chromatographic investigation of the n-hexane fraction

The *n*-hexane fraction (20 g) was subjected to silica gel column (800 g, 130 x 5 cm) using *n*-hexane:EtOAc gradient. Four subfractions were obtained HFI to HFIV. HF-II (4 g, *n*-hexane:EtOAc 95:5) was chromatographed over silica gel column using *n*-hexane:EtOAc gradient elution to afford compounds **1** (50 mg, colorless fine needles) and **2** (50 mg, white crystals). HF-IV (4.4 g, *n*-hexane:EtOAc 85:15) was subjected to silica gel column chromatography (120 g, 50 x 5 cm) eluted with *n*-hexane:EtOAc gradient, where compounds **3** (90 mg, white crystalline needles) and **4** (50 mg, white crystalline needles) were isolated.

Chromatographic investigation of the $CHCl_3$ fraction

The $CHCl_3$ fraction (12 g) was chromatographed over silica gel column (500 g, 100 x 5 cm) using $CHCl_3$:MeOH gradient elution. Three subfractions were obtained CF-I to CF-III. Silica gel column chromatography (150 g, 50 x 3 cm) of CF-II (4 g, $CHCl_3$:MeOH 95:5) using $CHCl_3$:MeOH gradient gave **5** (70 mg, colorless crystal), **6** (25 mg, white granular powder), and **7** (30 mg, yellowish white crystals). CF-III (2.5 g $CHCl_3$:MeOH 9:1) was subjected to silica gel column chromatography (100 g, 50 x 3 cm) eluted with $CHCl_3$:MeOH gradient elution to afford compounds **8** (25 mg, yellow amorphous powder), **9** (20 mg, yellow amorphous powder), and **10** (20 mg, colorless needles).

Chromatographic investigation of the EtOAc fraction

EtOAc fraction (17 g) was subjected to silica gel column chromatography (600 g, 100 x 5 cm) eluted with $CHCl_3$:MeOH gradient to give four subfractions EF-I to EF-IV. These sub-fractions were further chromatographed over silica gel column, eluted with $CHCl_3$:

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MeOH to afford compounds **11** (50 mg, yellow amorphous powder), **12** (70 mg, yellow amorphous powder), and **13** (30 mg, yellow amorphous powder).

Chromatographic investigation of the n-BuOH fraction

Silica gel column chromatography (500 g, 100 x 5 cm) of *n*-BuOH fraction (12 g) using $CHCl_3$:MeOH gradient afforded two subfractions. They were further chromatographed over silica gel column, eluted with $CHCl_3$:MeOH gradient to give compounds **14** (20 mg, yellow amorphous powder) and **15** (40 mg, yellow amorphous powder).

Spectral data

α-Amyrin (**1**): Colorless fine needles (50 mg, acetone), m.p. 184-186 °C. R_f . 0.29 and 0.61 (S_1 & S_2). IR (KBr): γ_{max} 3455 (OH), 2940, 1617 (C=C), 1454, 1385, 1062 cm^{-1}.

Hexacosanol (**2**): White crystals (50 mg, acetone), m.p. 78-80 °C. R_f: 0.27 and 0.48 (S_1 & S_2). IR (KBr): γ_{max} 3275, 2895, 1454, 1055 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ_H 3.64 (2H t, *J* = 6.9 Hz, H-1), 1.2-1.5 (48 H, m, $(CH_2)_{24}$), 0.87 (3H, t, *J* = 6.8 Hz, H-26). EIMS (rel. int.): *m/z* 382 $[M]^+$ (3.9), 364 $[M-H_2O]^+$ (100), 336 (25), 282 (1.3), 237 (5.2), 210 (7), 154 (5.4), 125 (20), 111 (25), 97 (60.4), 83 (72.6), 69 (61.5), 56 (75.7).

Stigmasterol (**3**)*:* White crystalline needles (acetone, 90 mg), m.p. 170 °C. R_f: 0.17 and 0.33 (S_1 & S_2). ^1H NMR (400 MHz, $CDCl_3$): δ_H 5.33 (1H, brs, H-6), 5.15 (1H, dd, *J* = 8.5, 15 Hz, H-22), 5.01 (1H, dd, *J* = 5, 15 Hz, H-23), 3.52 (1H, m, H-3), 1.00 (3H, s, H-19), 0.90 (3H, d, *J* = 6.3 Hz, H-21), 0.82 (6H, d, *J* = 7.4 Hz, H-26, 27), 0.79 (3H, t, *J* = 7.4 Hz, H-29), 0.67 (3H, s, H-18). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C 140.7 (C-5), 138.3 (C-22), 129.2 (C-23), 121.7 (C-6), 71.8 (C-3), 56.8 (C-17), 56.0 (C-14), 51.2 (C-25), 50.1 (C-9), 45.8 (C-24), 42.3 (C-12), 40.5 (C-13), 39.7 (C-4), 36.5 (C-10, 20), 37.2 (C-1), 31.9 (C-8), 31.6 (C-7), 29.1 (C-2), 28.2 (C-16), 24.3 (C-15), 23.0 (C-28), 21.2 (C-11), 19.8 (C-27,19), 19.0 (C-26), 18.8 (C-21), 12.2 (C-29), 12.0 (C-18).

Oleanolic acid (**4**): White crystalline needles (50 mg, acetone), m.p. 271-273 °C. R_f: 0.16 and 0.25 (S_1 & S_2). IR (KBr): γ_{max} 3649, 2946, 1619, 1457, 1374, 1028, 1016 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ_H 5.26 (1H, t, *J* = 3.5 Hz, H-12), 3.23 (1H, dd, *J* = 11.2, 4.4 Hz, H-3), 2.2 (1H, dd, *J* = 13.2, 3.6 Hz, H-18), 1.37 (3H, s, H-27), 1.25 (3H, s, H-23), 1.08 (6H, s, H-24, 30), 0.99 ((3H, s, H-25), 0.93(3H, s, H-29), 0.87 (1H, d, *J* = 6.4 Hz, H-5).

β-Sitosterol-3-O-β-glucopyranoside (**5**): Colorless crystal (70 mg, MeOH), m.p. 278 °C. R_f: 0.29 and 0.66 (S_3 & S_4). ^1H NMR data (400 MHz, DMSO-d_6): δ_H 5.28 (1H, brs, H-6), 4.45 (1H, brs, H-3), 4.19 (1H, d, *J* = 7.5 Hz, H-1´), 2.7-3.35 (5H, m, sugar protons), 0.92 (3H, brs, H-21), 0.85 (6H, d, *J* = 6.5 Hz, H-26, 27), 0.77 (3H, t, *J* = 7.0 Hz, H-29), 0.74 (3H, s, H-19), 0.60 (3H, s, H-18). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 140.4 (C-5), 121.2 (C-6), 100.8 (C-1`), 78.9 (C-5`), 78.6 (C-3`),73.5 (C-2`), 70.1 (C-4`), 67.9 (C-3), 61.1 (C-6`), 56.2 (C-14), 55.4 (C-17), 49.6 (C-9), 45.1 (C-24), 41.9 (C-13), 40.1 (C-12), 38.9 (C-4), 36.8 (C-10), 36.2 (C-1), 36.1 (C-22), 35.5 (C-20), 33.3 (C-7), 31.4 (C-8), 29.8 (C-2), 29.3 (C-25), 28.7 (C-16), 25.4 (C-23), 24.4 (C-15), 23.9 (C-28), 20.6 (C-11, 27), 19.7 (C-26), 19.1 (C-19), 18.9 (C-21), 11.7 (C-29).

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β-Sitosterol-3-O-β-glucouronopyranoside (**6**): White granular powder (25 mg, methanol). R_f: 0.29 and 0.66 (S_3 & S_4). ^{1}H NMR data (500 MHz, DMSO-d_6): δ_H 8.34 (1H, s, 6'-OH), 5.31 (1H, brs, H-6), 4.22 (1H, brs, H-3), 4.21 (1H, d, *J* = 7.5 Hz, H-1´), 2.60-3.49 (4H, m, sugar protons), 0.94 (3H, brs, H-19), 0.88 (3H, d, *J* = 6.5 Hz, H-21), 0.78 (6H, t, *J* = 7.0 Hz, H-26, 27), 0.77 (3H, s, H-29), 0.63 (3H, s, H-18). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 175.5 (C-6`), 140.9 (C-5), 121.7 (C-6), 101.1 (C-1`), 77.5 (C-3`), 77.1 (C-3), 73.9 (C-5`), 70.5 (C-2`), 70.2 (C-4`), 56.6 (C-14), 55.8 (C-17), 50.0 (C-9), 45.6 (C-24), 42.3 (C-13), 40.0 (C-12), 38.2 (C-4), 37.3 (C-1), 36.7 (C-10), 36.6 (C-22), 35.9 (C-20), 31.9 (C-8), 31.8 (C-7), 29.4 (C-25), 29.0 (C-16), 28.9 (C-2), 25.8 (C-23), 24.3 (C-15), 23.0 (C-28), 21.0 (C-11), 20.1 (C-27), 19.5 (C-19), 19.4 (C-21), 19.1 (C-26), 12.2 (C-29), 12.1 (C-18).

P-Hydroxy benzoic acid (**7**): Yellowish white crystals (30 mg, MeOH), m.p 213-214 °C. R_f: 0.27 and 0.48 (S_3 & S_4). UV (MeOH): λ_{max} 252 nm. IR (KBr): γ_{max} 3460, 3381, 1574, 894, 842, 770 cm^{-1}. ^{1}H NMR data (500 MHz, DMSO-d_6): δ_H 12.1 (1H, brs, COOH), 8.5 (1H, brs, 4-OH), 7.92 (2H, d, *J* = 8.6 Hz, H-2, 6), 6.81 (2H, d, *J* = 8.6 Hz, H-3, 5). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 167.8 (C-7), 153.4 (C-4), 131.6 (C-2, 6), 117.2 (C-1), 112.99 (C-3, 5). EIMS (rel. int.): *m/z* 138 (96.7%) $[M]^+$, 119 (100%), 86 (25.2%), 77 (1.7%).

Kaempferol (**8**): Yellow amorphous powder (25 mg, MeOH). R_f: 0.12 and 0.25 (S_3 & S_4). UV (MeOH) : λ_{max} 265, 355; +NaOMe: 273, 400; +$AlCl_3$: 281, 440; +$AlCl_3$/HCl: 281, 433; +NaOAc: 281, 370; +NaOAc/H_3BO_3: 269, 360 nm.

Quercetin (**9**): Yellow amorphous powder (20 mg, MeOH). R_f: 0.10 and 0.15 (S_3 & S_4). UV (MeOH): λ_{max} 270, 368; +NaOMe: 295, 417; +$AlCl_3$: 290, 453; +$AlCl_3$/HCl: 277, 400; +NaOAc: 286, 400; +NaOAc/H_3BO_3: 278, 385 nm.

Methyl gallate (**10**): Colorless needles (20 mg, MeOH), m.p. 201-203 ^{o}C. R_f: 0.42 and 0.65 (S_3 & S_4). UV (MeOH): λ_{max} 215 and 271 nm. ^{1}H NMR data (500 MHz, DMSO-d_6): δ_H 8.46 (1H, s, OH), 7.20 (2H, brs, H-2, 6), 3.65 (3H, s, OCH_3). EIMS (rel. int.): *m/z* 184 (6.2%), 161 (11.7%), 125 (21%), 109 (2.1%), 93 (3%), 77 (14%).

Quercetin-3-O-β-D-glucopyranoside (**11**): Yellow amorphous powder (50 mg, MeOH). R_f: 0.27 and 0.4 (S_4 & S_5). UV (MeOH): λ_{max} 257, 348; +NaOMe: 275, 388; +$AlCl_3$: 275, 431; +$AlCl_3$/HCl: 268, 394; +NaOAc: 270, 360; +NaOAc/H_3BO_3: 261, 370 nm. ^{1}H NMR (500 MHz, DMSO-d_6): δ_H 12.50 (1H, brs, 5-OH), 7.62 (1H, brs, H-2`), 7.50 (1H, d, *J* = 7.6 Hz, H-6`), 6.78 (1H, d, *J* = 7.6 Hz, H-5`), 6.34 (1H, brs, H-8), 6.12 (1H, brs, H-6), 5.31 (1H, d, *J* = 7.6 Hz, H-1``), 3.31-3.81 (5H, m, sugar protons).

Kaempferol-3-O-β-D-glucopyranoside (**12**): Yellow amorphous powder (70 mg, MeOH). R_f: 0.21 and 0.30 (S_4 & S_5). UV (MeOH): λ_{max} 260, 350; +NaOMe: 278, 408; +$AlCl_3$: 278, 400; +$AlCl_3$/HCl: 278, 400; +NaOAc: 273, 355; +NaOAc/H_3BO_3: 262, 357 nm. ^{1}H NMR (500 MHz, DMSO-d_6): δ_H 12.50 (1H, brs, 5-OH), 7.95 (2H, d, *J* = 8.5 Hz, H-2`, 6`), 6.84 (2H, d, *J* = 8.5 Hz, H-3`, 5`), 6.41 (1H, brs, H-8), 6.10 (1H, brs, H-6), 5.3 (1H, d, *J* = 8.0 Hz, H-1``), 3.3-3.8, (5H, m, sugar protons). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 177.8 (C-4), 160.4 (C-7), 157.4 (C-4`), 156.9 (C-9), 156.6 (C-2), 133.0 (C-3), 132.0 (C-5), 131.4

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(C-2`, 6`), 120.8 (C-1`), 115.6 (C-3`, 5`), 104.0 (C-10), 101.0 (C-1``), 99.0 (C-6), 94.1 (C-8), 73.0 (C-5``), 72.9 (C-3``), 71.0 (C-2``), 70.0 (C-4``), 63.0 (C-6``).

Kaempferol 3-O-rhamnoside (**13**): Yellow amorphous powder (30 mg, MeOH). R_f: 0.17 and 0.28 (S_4 & S_5). UV (MeOH): λ_{max} 262, 352; +NaOMe: 268, 408; +$AlCl_3$: 278, 400; +$AlCl_3$/HCl: 278, 400; +NaOAc: 273, 375; +NaOAc/H_3BO_3: 266, 357 nm. ^{1}H NMR (500 MHz, DMSO-d_6): δ_H 12.58 (1H, brs, 5-OH), 7.95 (2H, d, *J* = 8.5 Hz, H-2`, 6`), 6.83 (2H, d, *J* = 8.5 Hz, H-3`, 5`), 6.40 (1H, brs, H-8), 6.16 (1H, brs, H-6), 5.38 (1H, d, *J* = 8.0 Hz, H-1``), 3.07-4.00 (4H, m, sugar protons), 0.88 (3H, d, *J* = 6.8 Hz, H-6``).

Kaempferol-3-O-rutinoside (**14**): Yellow amorphous powder (20 mg, MeOH). R_f: 0.25 and 0.40 (S_4 & S_5). UV (MeOH): λ_{max} 255, 350; +NaOMe: 265, 410; +$AlCl_3$: 265, 400; +$AlCl_3$/HCl: 265, 400; +NaOAc: 267, 365; +NaOAc/H_3BO_3: 257, 356 nm. ^{1}H NMR (500 MHz, DMSO-d_6): δ_H 8.00 (2H, d, *J* = 8.8 Hz, H-2`, 6`), 6.85 (2H, d, *J* = 8.8 Hz, H-3`, 5`), 6.34 (1H, brs, H-8), 6.12 (1H, brs, H-6), 5.42 (1H, d, *J* = 7.6 Hz, H-1``), 4.29 (1H, brs, H-1```), 3.31-3.80 (9H, m, sugar protons), 1.19 (3H, d, *J* = 6.8 Hz, H-6```).

Quercetin-3-O-rutinoside (**15**): Yellow amorphous powder (40 mg, MeOH). R_f: 0.17 and 0.28 (S_4 & S_5). UV (MeOH): λ_{max} 256, 353; +NaOMe: 278, 396; +$AlCl_3$: 276, 430; +$AlCl_3$/HCl: 276, 403; +NaOAc: 277, 385; +NaOAc/H_3BO_3: 267, 370. ^{1}H NMR (500 MHz, DMSO-d_6): δ_H 12.59 (1H, brs, 5-OH), 7.55 (1H, brd, *J* = 7.6 Hz, H-6`), 7.51 (1H, brs, H-2`), 6.84 (1H, d, *J* = 7.6 Hz, H-5`), 6.37 (1H, brs, H-8), 6.18 (1H, brs, H-6), 5.07 (1H, d, *J* = 8.0 Hz, H-1``), 4.31 (1H, brs, H-1```), 3.30-3.80 (4H, m, sugar protons), 0.98 (3H, d, *J* = 6.5 Hz, H-6```). ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 177.4 (C-4), 159.1 (C-7), 156.7 (C-9), 156.5 (C-2), 148.5 (C-4`), 144.8 (C-3`), 133.3 (C-3), 132.3 (C-5), 121.6 (C-6`), 121.2 (C-1`), 116.3 (C-2`), 115.3 (C-5`), 104.0 (C-10), 101.2 (C-1``), 100.8 (C-1```), 98.7 (C-6), 93.7 (C-8), 76.5 (C-3``), 75.9 (C-5``), 74.7 (C-2``), 71.9 (C-4```), 70.6 (C-3```), 70.2 (C-4``), 70.0 (C-2```), 68.3 (C-5```), 67.0 (C-6``), 17.8 (C-6```).

Acid hydrolysis

A solution of the isolated glycoside (5 mg in 10 mL MeOH) was treated with 5% H_2SO_4 (1.5 mL) and refluxed on a boiling water bath for 3 hr. The aglycone was extracted with EtOAc, concentrated under reduced pressure, purified on Sephadex LH-20 column using MeOH and identified by co-TLC with an authentic sample. The sugars in the aqueous layer were identified by co-PC (paper chromatography) with authentic materials using solvent system (S_6) (Chaturvedulaet al, 2011).

Antimicrobial activity

Preparation of the extracts

The air dried powdered plant (200 g) was extracted with MeOH 70 % and concentrated under reduced pressure. The concentrated extract was suspended in distilled water, and partitioned between *n*-hexane, $CHCl_3$, and EtOAC. Each extract was concentrated under reduced pressure and sterilized by overnight UV-irradiation and sterility checked by plating the reconstituted extract on nutrient agar. 0.5 g of each extract was reconstituted in 10 mL of

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dimethyl formamide (DMF). DMF served as a negative control because it has no observed antibacterial activity against the tested organisms.

Microbial strains

The organisms used in this study were *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Salmonella typhimurium*, and *Candida albicans*. They were obtained from the Microbiology Department, Faculty of Medicine, Assiut University.

Antimicrobial activity

The procedure was carried out as previously described (Bonev et al, 2008). The antibacterial and antifungal activities were evaluated using the agar plate diffusion assay. Bacteria were grown on nutrient agar (Oxoid, England) and fungi on Sabouraud's glucose agar (Oxoid, England). Susceptibility discs (5.5 mm) were impregnated with a solution of each extract at concentration of 500 µg/disc. The discs were dried and placed on agar plates inoculated with the tested bacterial strains: *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, and *Salmonella typhimurium*, and the fungal strain: *Candida albicans*. Each plate was inoculated with a single organism and the test was run in duplicates. The plates were incubated at 37 ºC and checked for inhibition zones after 24 hr for bacteria and after 48 hr for fungi. Ampicillin trihydrate and miconazole were used as positive reference standards for antibacterial and antifungal activities, respectively. The bacterial growth inhibition zone diameter (IZD) was measured to the nearest mm.

Determination of the minimal inhibitory concentration (MIC)

The MIC was determined by a twofold dilution method against the tested microorganisms (Bonev et al, 2008). Each extract was dissolved in DMF according to their respective known weights. The resulted solutions were serially diluted with DMF and were added to disc diffusion method on nutrient agar medium at concentrations 100, 200, and 500 µg/disc. The minimum inhibition concentration (MIC) at which no visible growth was observed and defined as the MIC, which was expressed in mm.

Pharmacological studies

Preparation of the extracts

Weighed amount of each extract (200 and 400 mg/Kg) was suspended in distilled H_2O using Tween 80 (2 % v/v) and different dose levels of theses extracts were interpretoneally injected in different groups of mice.

Experimental animals

Male albino mice (20-25 g body weight) and adult male albino rats (120-140 g body weight) of both sexes were used. The animals were housed under standardized environmental conditions in the pre-clinical Animal House, Pharmacology Department, Faculty of Medicine, Assuit University. They were fed with standard diet and water.

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Anti-inflammatory activity

The anti-inflammatory activity of the tested extracts was evaluated in adult albino rats by yeast-induced paw edema method according to the published procedures (Sawadogo et al, 2006). The percentage of edema inhibition (% of change) was calculated.

Analgesic activity

The analgesic activity of the tested extracts was evaluated in ten groups of adult albino mice (20-25 g) by acetic acid-induced writhing method (Sawadogo et al, 2006; Ezeja et al, 2011). Number of writhes (abdominal contractions) in all animals was counted and the results were recorded.

Antipyretic activity

The antipyretic activity of the tested extracts was screened in adult albino rats by using yeast-induced hyperpyrexia method (Sawadogo et al, 2006). Rectal temperature of each rat was recorded after 1, 2, 3, and 4 hr from administration of tested fractions.

Brine shrimp lethality test

Activity against brine shrimp, *Artemia salina* was determined as previously outlined (Meyer et al, 1982).

Statistical analysis

Data were analyzed using student's "*t*" test and the values were expressed as mean± S.E. (n = 6 animals).

Result and discussion

Preliminary phytochemical screening of alcoholic extract of the whole plant indicated the presence of carbohyrates and/or glycosides, unsaturated sterols and/or triterpenes, tannins, and flavonoids. These secondary metabolites are known to possess various pharmacological effects. GC-MS study of the fatty acid methyl esters of the *n*-hexane fraction revealed the presence of four major fatty acids; palmitic, octadecanoic, 6-octadecenoic, and myristic acids. Chemical investigation of the different fractions afforded the isolation of fifteen compounds (Figure 1). They were identified as α-amyrin (**1**) (Abdel-Monem et al, 2008), hexacosanol (**2**) (Rizk et al., 1980), stigmasterol (**3**) (Greca et al, 1990), oleanolic acid (**4**) (Maillard et al, 1992; Yan-Ping & Zhong-Jian, 1997), *β*- sitosterol-3-O-*β*-D-glucopyranoside (**5**) (Jassbi, 2006), *β*-sitosterol-3-*β*-O-D-glucouronopyranoside (**6**) (Stachulski et al, 1998), *p*-hydroxy benzoic acid (**7**) (Gerothanassis et al, 1998), kaempferol (**8**) (Dumkow et al, 1973), quercetin (**9**) (Dumkow et al, 1973; Sikorska & Matlawska, 2000), methyl gallate (**10**) (Yoshida et al, 1994), quercetin-3-O-*β*-D-glucopyranoside (**11**) (Dumkow et al, 1973; Sikorska & Matlawska, 2000), kaempferol-3-O-*β*-D-glucopyranoside (**12**) (Dumkow et al, 1973), kaempferol-3-O-rhamnoside (**13**) (Jassbi, 2006), kaempferol-3-O-rutinoside (**14**) (Jassbi, 2006), and rutin (**15**) (Jassbi, 2006) by comparison of their physicochemical and sep-

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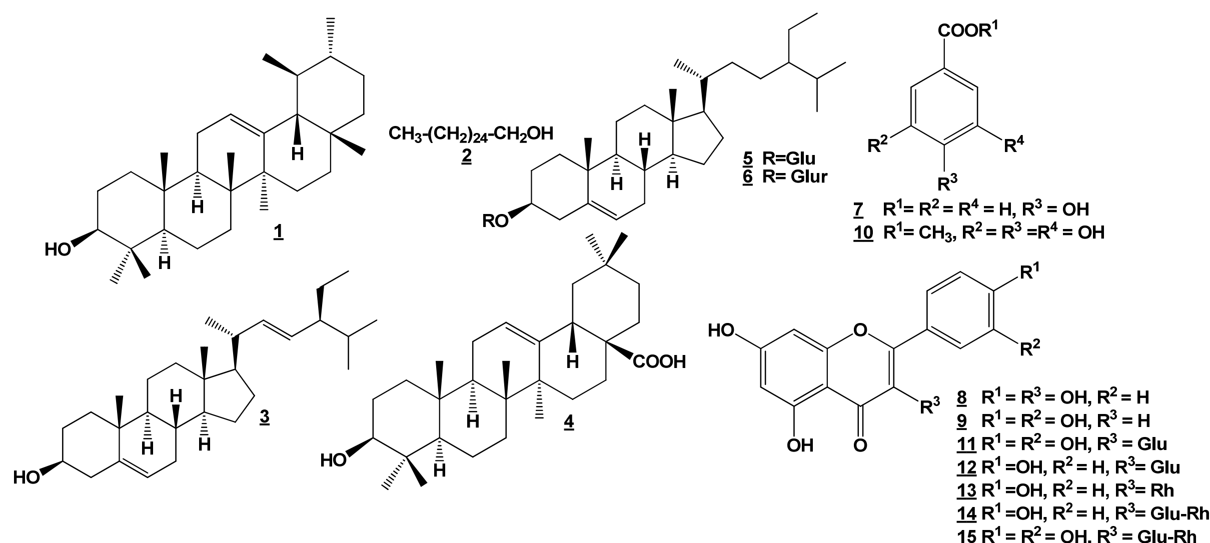

Figure 1: Structures of the isolated compounds.

Table 1. Results of antimicrobial activity.

Extract	Bacterial strains								Fungal strain	
	S. aureus		*B. cereus*		*Salmonella typhimurium*		*E. coli*		*Candida albicans*	
	IZ[a]	MIC[b]	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC
n-Hexane	7	11	10	12	7	10	7	15	6	10
$CHCl_3$	5	5	7	7	5	6	6	8	2	8
EtOAc	15	12	12	13	10	11	8	18	8	16
MeOH	16	10	14	12	12	10	9	16	9	18
Ampicillin[c] trihydrate	11	13	15	14	13	11	10	10	-	-
Miconazole[d]	-	-	-	-	-	-	-	-	10	17

[a] Inhibition zone diameter
[b] Minimal inhibitory concentration
[c] Positive control for antibacterial activity
[d] Positive control for antifungal activity

Table 2: Results of anti-inflammatory activity.

Extract	Dose (mg/kg)	Thickness of the right hind paw (mm)			
		0 hr	1 hr	3 hr	5 hr
		(mean ± SE). n=6			
Control negative	0	3.20 ± 0.17	6.60 ± 0.19	6.80 ± 0.17	7.30 ± 0.25
Indomethacin	8	3.30 ± 0.14	5.60 ± 0.18***	3.70± 0.12*	3.50 ± 0.24
MeOH	200	4.10 ± 0.12	4.50 ± 0.23	3.80± 0.14	4.40 ± 0.21
	400	3.40 ± 0.30	5.60 ± 0.18***	4.50 ± 0.18*	4.10 ± 0.12*
n-Hexane	200	4.70 ± 0.11	5.50 ± 0.26	6.15 ± 0.23***	5.90 ± 0.19***
	400	3.60 ± 0.31	6.00 ± 0.27***	5.50 ± 0.22***	5.5 ± 0.22***
$CHCl_3$	200	4.16 ± 0.33	5.96 ± 0.23	6.80 ± 0.23	6.56 ± 0.31**
	400	3.30 ± 0.23	6.00 ± 0.27***	6.40 ± 0.18***	6.00 ± 0.15***
EtOAc	200	3.50 ± 0.31	4.90 ± 0.67	3.80 ± 0.31**	4.90 ± 0.33*
	400	3.30 ± 0.33	4.70 ± 0.12	3.40 ± 0.19*	3.9 ± 0.20*

S.E= standard error. n = number of animals. Data are expressed as mean±S.E, n = 6. Differences with respect to the control group were evaluated using the student's *t*-test (*P<0.05, **P<0.01, ***P<0.005).

ctral data with those in the literature as well as co-chromatography with authentic samples. The different extracts of *Euphorbia peplus* L. exhibited antimicrobial activity against the tested strains. EtOAc and MeOH extracts showed high activity against *Staphylococcus aureus, Bacillus cereus, Salmonella typhimurium, Escherichia coli*, and *Candida albicans* (Table 1). The observed activities may be due to their contents of phenolics, triterpenes, and sterols. Anti-inflammatory activity of the different extracts was measured against yeast-induced edema (Table 2). The MeOH and EtOAc extracts at doses of 200 and`400 mg/kg give potent

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Table 3. Results of analgesic activity.

Extract	Dose (mg/kg)	Writhing response in mice (mean ± S.E.), n = 6		
		5 min	10 min	15 min
Control	-	45.17 ± 0.55	39.20 ± 0.56	38.89 ± 0.53
ASA	150	17.16 ± 0.71**	15.34 ± 0.57**	13.45 ± 0.44**
MeOH	200	35.67 ± 0.24**	30.57 ± 0.55**	28.45 ± 0.56**
	400	33.45 ± 0.57**	29.24 ± 0.38**	26.18 ± 0.55**
n-Hexane	200	28.45 ± 0.53**	27.34 ± 0.56**	25.67 ± 0.34**
	400	20.26 ± 0.57**	18.29 ± 0.35**	15.64 ± 0.35**
$CHCl_3$	200	33.00 ± 0.56**	30.00 ± 0.34**	29.67 ± 0.45**
	400	29.34 ± 0.56**	25.55 ± 0.34**	21.66 ± 0.76**
EtOAc	200	29.55 ± 0.53**	25.77 ± 0.56**	19.67 ± 0.33**
	400	24.22 ± 0.55**	20.29 ± 0.35**	18.64 ± 0.56**

S. E = standard error; n = number of animals; Data are expressed as mean ±SEM, n = 6; ASA= Acetyl salicylic acid; Differences with respect to the control group were evaluated using student's *t*-test (*P<0.05, **P<0.01).

Table 4. Results of antipyretic activity.

Group	Dose (mg/kg)	Average rectal temperature (°C) ± S.E., n = 6			
		1 hr	2 hr	3 hr	4 hr
Control	0	38.73 ± 0.05	39.00 ± 0.08	38.93 ± 0.07	38.65 ± 0.18
Indomethacin	8	37.16 ± 0.07**	36.34 ± 0.11**	35.45 ± 0.18**	35.62 ± 0.14**
MeOH	200	38.17 ± 0.07	36.38 ± 0.13**	36.33 ± 0.14**	35.85 ± 0.24**
	400	38.37 ± 0.06	36.44 ± 0.22**	36.27 ± 0.30**	35.37 ± 0.25**
n-Hexane	200	38.17 ± 0.09	37.22 ± 0.13**	36.33 ± 0.22**	35.20 ± 0.11**
	400	38.42 ± 0.06	36.40 ± 0.21**	35.79 ± 0.29**	35.13 ± 0.09**
$CHCl_3$	200	38.42 ± 0.09	38.00 ± 0.06*	37.02 ± 0.05**	35.20 ± 0.11**
	400	38.35 ± 0.13	37.10 ± 0.16*	36.30 ± 0.25**	35.33 ± 0.08**
EtOAc	200	38.78 ± 0.06	37.60 ± 0.18**	37.30 ± 0.09**	37.23 ± 0.08**
	400	38.4 ± 0.10	36.90 ± 0.10*	36.82 ± 0.13**	35.78 ± 0.17**

S.E. = standard error; n = number of animals; Data are expressed as mean ± S.E., n = 6; Differences with respect to the control group were evaluated using student's *t*-test (*P<0.05, **P<0.01).

Table 5. Results of the brine shrimp lethality test.

Extract	Mortality %	
	100 µg/mL	200 µg/mL
MeOH	25	35
n-Hexane	20	40
$CHCl_3$	40	88
EtOAc	10	10

anti-inflammatory activity compared with indomethacin. While, *n*-hexane and $CHCl_3$ extracts showed weak activity. All extracts were subjected to testing their analgesic activity using the acetic acid-induced writhing method (Table 3). Significant protection against writhing was observed in the different extracts (400 mg/kg for each) compared with acetyl salicylic acid (ASA) (150 mg/kg). The tested extracts showed antipyretic activity at doses 200 and 400 mg/kg for each. They control the hyperthermia for 4 hr without decrease in activity (Table 4). In the brine shrimp bioassay, the $CHCl_3$ extract showed strong activity represented by the highest percent of mortality (Table 5). These results may be attributed to the presence of certain terpenoids in the $CHCl_3$ extract which have a reported cytotoxic effect (Zheng et al, 1998).

Fifteen compounds were isolated and elucidated from *Euphorbia peplus* L. Compounds **1**, **4**, **5**, **7**, **10**, and **13-15** were reported from the plant for the first time and **6** was isolated

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for the first time from the family. The EtOAc and MeOH extracts showed high antimicrobial and anti-inflammatory activities, while the $CHCl_3$ extract exhibited strong cytotoxic activity.

Conflict of interest

There is no conflict of interest associated with the authors of this paper.

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