Research Article

PHYTOCHEMICAL AND BIOLOGICAL INVESTIGATION OF *URTICA URENS* L. GROWING IN EGYPT

Hosam M. El-Seadawy, Kamilia A. Abo El-Seoud, Amal M. Kabbash, Mona El-Aasr and Ghada I. Attia *

Department of Pharmacognosy, Faculty of Pharmacy, Tanta University, Tanta (31527), Egypt

*Corresponding Author Email: ghadaismalattia18@gmail.com

Article Received on: 27/12/17 Approved for publication: 22/01/18

DOI: 10.7897/2230-8407.0915

ABSTRACT

Phytochemical investigation of *Urtica urens* L. aerial parts resulted in isolation of kaempferol 3,7-di-*O*- α -L-rhamnoside (kaempferitrin), tryptophan and adenosine for the first time from this species; in addition to β -sitosterol, β -sitosterol-3-*O*- β -D-glucoside, scopoletin, quercetin and succinic acid. The chemical structure of the compounds was determined using different spectroscopic techniques including UV, IR, EI-MS, ^1H NMR and ^{13}C NMR. Antioxidant, cytotoxic and anti-hyperglycemic activities of *Urtica urens* L. family Urticaceae were investigated. By using DPPH radical scavenging method and quercetin as standard antioxidant compound, the methanol extract of the aerial parts showed a significant antioxidant activity. Cytotoxic activity using SRB assay method was carried out on the total methanol extract in addition to four different fractions (petroleum ether, methylene chloride, ethyl acetate and *n*-butanol) against four different cell lines. The ethyl acetate and the *n*-butanol fractions have the highest cytotoxic activity against HEPG2 and PC3 cell lines with percent inhibition 74% and 87% respectively in the single dose experiment. The results showed that the ethyl acetate fraction is more potent than doxorubicin against HEPG2 cell line and the *n*-butanol fraction is less potent than doxorubicin against PC3 cell line. *Urtica urens* L. aerial parts methanol extract showed anti-diabetic activity and improved other biochemical and histopathological parameters in STZ-induced diabetic rats compared to glibenclamide as standard.

Keywords: *Urtica urens*, kaempferitrin, scopoletin, HEPG2, PC3, anti-diabetic, STZ-induced diabetic rats

INTRODUCTION

Urtica urens L. is a member of Family Urticaceae which includes about 48 genera and 1050 tropical and warm temperate species¹. It's an annual herbaceous shrubs, native of Europe and has become naturalized throughout North America, Africa, Asia, Australia and South America². *U. urens* L. is one species of 3 genera grows in Egypt as wild weeds in the cultivated lands, around the River Nile, Mediterranean regions and Isthemic desert³. This plant is a rich source of phenolic compounds⁴. It is widely used as folk medicine⁵ and exhibited anti-nociceptive⁶, antioxidant⁷ and hepatoprotective effects⁸.

Beside the afore mentioned data, no previous investigations of phytochemical and biological activities of *U. urens* L. were carried out in Egypt. Therefore, the present study was undertaken to investigate various biological activities of *U. urens* L. aerial parts methanol extract and its different fractions. Antioxidant, cytotoxic activity against different cell lines, anti-hyperglycemic activity and other biochemical parameters in STZ-induced diabetic rats were studied.

MATERIALS AND METHODS

Plant materials

The aerial parts of *Urtica urens* L. were collected in November 2013 from the cultivated lands of potatoes and clover crops in Al Qurashiyyah village, Al Santah, El-Gharbia governorate, Egypt. The plant was kindly identified by Prof. Kamal Hussien Shaltout, Botany Department, Faculty of Science, Tanta University. A voucher specimen was deposited at the herbarium of Faculty of Pharmacy, Tanta University, Egypt. The aerial parts were dried

in shade, reduced to powder and stored in tightly closed containers.

Experimental animals

Adult male albino rats with an average body weight of 180-200 g were used. The study guidelines established by the National Institute of Health Guide for the Care and Use of Laboratory Animals were applied.

Cell lines for cytotoxic activity

Liver carcinoma (HEPG2), Prostate carcinoma (PC3), Breast carcinoma (MCF-7), and colon carcinoma (HCT) cell lines were obtained from the American type culture collection (ATCC).

Chromatographic materials and chemicals

Silica gel (70-230 mesh) from Fluka; Sephadex LH-20 from Sigma-Aldrich chemical Co., USA; Diaion HP-20 from Mitsubishi Chemical, Japan. Pre-coated TLC sheets silica gel G F₂₅₄ (E. Merck). 2,2-Diphenyl-1-picryl-hydrazyl (DPPH) from Sigma-Aldrich, Inc., USA. Quercetin from (Sigma Chemical Co., St. Louis, MO) was obtained for the investigation of antioxidant activity. Dimethyl sulfoxide (DMSO), trichloroacetic acid, sulforhodamine B (SRB), ethylenediaminetetraacetic acid buffer (EDTA), acetic acid were obtained from Sigma Chemical Co. St. Louis, MO and doxorubicin. HCl (positive control) from Sigma-Aldrich, Inc., USA for cytotoxic assay. Streptozotocin and sodium citrate buffer from Sigma Chemical Co. St. Louis, MO and Glibenclamide (Daonil 5 mg brand) manufactured by SANOFI pharmaceutical company, France for anti-hyperglycemic assay. Serum glucose level was determined by

Spinreact kits, Spain, Serum (AST) and (ALT) were assayed using kits provided by Human Gesellschaft für Biochemica und Diagnostica mbH, Germany, Serum urea and creatinine were determined using kits provided by Diamond diagnostics, Egypt and Commercial activity assay kits (Biodiagnostic, Egypt) were used to estimate antioxidant status, antioxidant enzymes.

Apparatus

GC/MS finnigan mat SSQ7000, USA, was used for fatty acid methyl esters analysis, melting point determination was carried out using Gallenkamp melting point apparatus, IR spectra were recorded using KBr disc using Jasco FT/IR-6100 spectrophotometer, UV spectra were recorded using Jasco UV/Vis spectrophotometer, V-530, Japan. EI/MS spectra were recorded on Thermo Scientific DSQ Single Quadrupole MS, USA. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance III 400 MHz Spectrometer, Germany. Chemical shifts were given on a δ (ppm) scale with tetramethylsilane (TMS) as an internal standard. An ELISA Processor II Microplate Reader was used for cytotoxic assessment, Shimadzu UV spectrophotometer, UV-1800, Japan was used for antioxidant assay and biochemical studies. Accu-Chek sensor comfort glucometer (China) and an intra-gastric tube were used for anti-hyperglycemic assay.

Extraction and isolation

The dried powder of *Urtica urens* L. aerial parts (5.5 kg) were extracted with methanol till exhaustion by cold maceration method. The total methanol extract was evaporated under reduced pressure to yield (700 g) residue, which was suspended in 50% aqueous methanol and successively fractionated with petroleum ether (40-60 °C), methylene chloride, ethyl acetate and *n*-butanol.

Petroleum ether extract (4 g) was saponified by boiling under reflux with 60 ml (10%) alcoholic KOH for 6 hrs. The alcohol was distilled off and the aqueous liquid left was diluted with 40 ml water and extracted several times with ether. The combined ethereal extract was evaporated under reduced pressure to give unsaponifiable residue (1.5 g). The alkaline aqueous solutions, remained were separately acidified with conc. HCl and the liberated fatty acids were extracted with ether. The combined ethereal extract was evaporated under reduced pressure to give fatty acids residue (0.2 g), which was dissolved in 25 ml methanol, 3 ml conc. sulfuric acid was added and refluxed for 2 hours. The methanol was distilled off and the residue was extracted by ether. The combined ethereal extract was evaporated under reduced pressure to give fatty acid methyl esters which were analyzed adopting GC-MS standard conditions.

The unsaponifiable matter was chromatographed on a silica gel column (ϕ 3 × 27 cm, 60 g) by gradient elution starting with pet. ether and the polarity were increased using methylene chloride then methanol to afford 8 fractions (fr. 1-8). Fr. 4 eluted with (pet. ether : methylene chloride = 30:70) was evaporated and recrystallized from methanol to yield compound 1 (68 mg). Compound 1 was obtained as white needle crystals; m.p 137-139°C; R_f =0.27 and 0.61 solvent system (pet. ether:CH₂Cl₂ = 3:7 and → CH₂Cl₂: MeOH = 9.5:0.5); IR (KBr) cm^{-1} : 3422, 2946, 2860, 1639, 1457, 1381, 960 and 876; ^1H NMR [CDCl₃, 400 MHz] δ : 5.28 (1H, d, J =4.8 Hz, H-6), 3.47 (1H, m, H-3), 0.94 (3H, s, H-19), 0.85 (3H, d, J =6.4 Hz, H-21), 0.78 (3H, t, J =7.2 Hz, H-29), 0.76 (3H, d, J =7.2 Hz, H-26), 0.74 (3H, d, J =6.8 Hz, H-27), 0.61 (3H, s, H-18) and 0.65-2.24 (m, H-1, 2, 4, 7-9, 11, 12, 14-17, 20, 22-25, 28); ^{13}C NMR [CDCl₃, 100 MHz] δ : 37.2 (C-1), 31.6 (C-2), 71.8 (C-3), 42.2 (C-4), 140.7 (C-5), 121.7 (C-6), 31.9 (C-7 and C-8), 50.1 (C-9), 36.1 (C-10), 21.0 (C-11), 39.7 (C-12), 42.3 (C-13), 56.7 (C-14), 24.3 (C-15), 28.2 (C-16), 56.1

(C-17), 11.8 (C-18), 19.3 (C-19), 36.5 (C-20), 18.7 (C-21), 33.9 (C-22), 26.1 (C-23), 45.8 (C-24), 29.1 (C-25), 19.8 (C-26), 19.0 (C-27), 23.0 (C-28) and 12.0 (C-29).

Methylene chloride fraction (6 g) was chromatographed on silica gel column (ϕ 3.5 × 46 cm, 240 g) using CH₂Cl₂ - MeOH gradient elution to afford 7 fractions. Fr. 4 eluted with (CH₂Cl₂:MeOH = 96:4), 688 mg was further chromatographed using silica gel column (ϕ 24 × 2.5 cm, 27 g) by gradient elution using CH₂Cl₂ - MeOH to afford 4 subfractions (fr. 4-1 to fr. 4-4). Fr. 4-3 eluted with (CH₂Cl₂:MeOH = 96:4) gave compound 2 (23 mg) after recrystallization from methanol. Compound 2 was obtained as yellow crystalline solid, m.p. 203-205 °C, R_f value 0.47 solvent system (toluene:ethyl acetate:formic acid = 5:4:1); UV λ_{max} MeOH: 228, 298, 345 nm; +NaOH: 230, 292, 392 nm; +AlCl₃: 229, 299, 345 nm; +AlCl₃/HCl: 229, 299, 345 nm; +NaOAc: 277, 391 nm; +NaOAc/Boric acid: 299, 347 nm; EI-MS [M]⁺ m/z at 192; ^1H NMR [acetone-*d*₆, 400 MHz] δ : 7.85 (1H, d, J =9.6 Hz, H-4), 7.20 (1H, s, H-5), 6.81 (1H, s, H-8), 6.19 (1H, d, J =9.6 Hz, H-3) and 3.91 (3H, s, OCH₃-6); ^{13}C NMR [acetone-*d*₆, 100 MHz] δ : 160.4 (C-2), 112.0 (C-3), 143.7 (C-4), 109.0 (C-5), 145.0 (C-6), 150.9 (C-7), 102.8 (C-8), 150.2 (C-9), 111.1 (C-10) and 55.8 (OCH₃-6).

Fr. 6 eluted with (CH₂Cl₂:MeOH = 94:6), 127 mg gave compound 3 after recrystallization from methanol. Compound 3 (18 mg) was obtained as white amorphous powder, m.p. 275-277 °C, R_f value 0.66 solvent system (CH₂Cl₂:MeOH = 9:1); ^1H NMR [DMSO-*d*₆, 400 MHz] δ : 5.33 (1H, bs, H-6), 4.22 (1H, d, J =7.6 Hz, H-1'), 3.63 (1H, m, H-3), 0.96 (3H, s, H-19), 0.91 (3H, d, J =6.4 Hz, H-21), 0.82 (3H, t, J =7.6 Hz, H-29), 0.83 (3H, d, J =6.8 Hz, H-26), 0.80 (3H, d, J =6.8 Hz, H-27), 0.66 (3H, s, H-18) and 0.66-2.15 (m, H-1, 2, 4, 7-9, 11, 12, 14-17, 20, 22-25, 28), 2.93-4.43 (m, Glc protons); ^{13}C NMR [DMSO-*d*₆, 100 MHz] δ : 37.3 (C-1), 29.7 (C-2), 77.3 (C-3), 39.3 (C-4), 140.9 (C-5), 121.6 (C-6), 31.8 (C-7), 31.9 (C-8), 50.0 (C-9), 36.6 (C-10), 21.0 (C-11), 38.7 (C-12), 42.3 (C-13), 56.6 (C-14), 24.3 (C-15), 28.2 (C-16), 55.9 (C-17), 12.1 (C-18), 19.4 (C-19), 35.9 (C-20), 19.0 (C-21), 33.8 (C-22), 25.9 (C-23), 45.6 (C-24), 29.1 (C-25), 20.1 (C-26), 19.5 (C-27), 23.0 (C-28), 12.2 (C-29), Glucose moiety: 101.2 (C-1'), 73.9 (C-2'), 77.2 (C-3'), 70.6 (C-4'), 77.2 (C-5') and 61.5 (C-6').

Ethyl acetate fraction (5.3 g) was chromatographed on silica gel column (ϕ 3.5 × 44 cm, 212 g) by gradient elution, starting with *n*-hexane and the polarity was increased using ethyl acetate then methanol to afford 3 fractions. Fr. 1 eluted with (*n*-hexane:ethyl acetate = 70:30 → 65:35 and → 60:40) was concentrated to give (112 mg) residue that was chromatographed on Sephadex LH-20 column (ϕ 1.5 × 25 cm, 15 g) using (100% MeOH) to afford 3 subfractions fr. 1-1 to fr. 1-3. Fr. 1-2 was crystallized from methanol to give compound 4 (43 mg). Compound 4 was obtained as yellow amorphous powder, m.p. 316-318 °C, R_f value 0.62 solvent system (CH₂Cl₂:MeOH = 9.5:0.5); UV λ_{max} MeOH: 256, 372 nm; +NaOH: 286, 426 nm; +AlCl₃: 271, 455 nm; +AlCl₃/HCl: 266, 302 (sh), 364, 430 nm; +NaOAc: 274, 322 (sh), 392 nm; +NaOAc/Boric acid: 261, 389 nm; ^1H NMR [CD₃OD, 400 MHz] δ : 7.63 (1H, d, J =1.6 Hz, H-2'), 7.53 (1H, dd, J =1.6 Hz, H-6'), 6.78 (1H, d, J =8.8 Hz, H-5'), 6.28 (1H, d, J =1.6 Hz, H-8) and 6.07 (1H, d, J =1.6 Hz, H-6); ^{13}C NMR [CD₃OD, 100 MHz] δ : 146.5 (C-2), 135.8 (C-3), 175.9 (C-4), 161.0 (C-5), 97.8 (C-6), 164.1 (C-7), 93.0 (C-8), 156.8 (C-9), 103.1 (C-10), 122.7 (C-1'), 114.5 (C-2'), 144.8 (C-3'), 147.3 (C-4'), 114.8 (C-5') and 120.2 (C-6').

Fr. 2 eluted with (*n*-hexane:ethyl acetate = 50:50 and → 45:55) was repeatedly crystallized using CH₂Cl₂ to yield compound 5 (30 mg). Compound 5 was obtained as colorless needle shaped crystals, m.p. 186-188 °C, R_f =0.51 solvent system

(CH₂Cl₂:MeOH = 8:2); IR KBr cm⁻¹: 3300-2500, 2646, 2538, 1693, 1419, 1310, 920, 803 and 637. Fr. 3 (533 mg) eluted with (ethyl acetate:MeOH = 99:1→ 98.5:1.5 and→ 98:2), was rechromatographed on silica gel column (ϕ 28 × 2.5 cm, 21 g) using gradient elution starting with 100% CH₂Cl₂ and the polarity was increased by using methanol and H₂O at different ratios (CH₂Cl₂:MeOH:H₂O = 9:1:0.1→ 8:2:0.2 and→ 7:3:0.5) to obtain 2 subfractions sub fr. 3-1 and sub fr. 3-2. Fr. 3-2 (92 mg) was rechromatographed on Sephadex LH-20 column (ϕ 1.5 × 25 cm, 15 g) using (100% MeOH) to give compound 6 (12 mg). Compound 6 was obtained as pale yellow needle crystals, m.p. 189-192°C, *R*_f=0.28 and 0.61 solvent system (CH₂Cl₂:MeOH:H₂O = 8:2:0.2→7:3:0.5) respectively; UV λ_{max} MeOH: 266,340 nm; +NaOH: 272,381 nm; +AlCl₃: 274, 300sh, 347,397 nm; +AlCl₃/HCl: 275, 294sh, 342, 396 nm; +NaOAc: 265, 350 nm; +NaOAc/Boric acid: 265, 343 nm; ¹HNMR [CD₃OD, 400 MHz] δ: 7.82 (2H, d, *J*=8.8 Hz, H-2' and H-6'), 6.96 (2H, d, *J*=8.8 Hz, H-3' and H-5'), 6.75 (1H, d, *J*=2 Hz, H-8), 6.49 (1H, d, *J*=2 Hz, H-6), 5.58 (1H, d, *J*=1.6 Hz, Rha-II-H-1'), 5.42 (1H, d, *J*=1.6 Hz, Rha-I-H-1), 1.28 (3H, d, *J*=6 Hz, Rha-II-C-5-Me), 0.96 (3H, d, *J*=5.6 Hz, Rha-I-C-5-Me) and 3.33-4.24 (m, sugar protons); ¹³CNMR [CD₃OD, 100 MHz] δ: 158.4 (C-2), 135.1 (C-3), 178.4 (C-4), 161.6 (C-5), 98.4 (C-6), 162.1 (C-7), 94.2 (C-8), 156.7 (C-9), 106.1 (C-10), 121.2 (C-1'), 130.5 (C-2', C-6'), 115.2 (C-3', C-5'), 160.3 (C-4'), 102.1 (Rha-I-C-1"), 70.5 (Rha-I-C-2"), 70.7 (Rha-I-C-3", Rha-II-C-3"), 71.7 (Rha-I-C-4"), 70.6 (Rha-I-C-5-Me), 16.2 (Rha-I-C-6"), 99.1 (Rha-II-C-1"), 70.3 (Rha-II-C-2"), 72.2 (Rha-II-C-4"), 69.8 (Rha-II-C-5-Me) and 16.6 (Rha-II-C-6").

n-Butanol fraction (30 g) was suspended in deionized water and loaded onto Diaion HP-20 column (ϕ 3 × 40 cm, 80 g), eluted sequentially with water followed by of 100% methanol and acetone. The 100% methanol eluate (3 g) was further subjected to a silica gel column (ϕ 3 × 45 cm, 120 g) using gradient elution starting with 100% CH₂Cl₂ and the polarity was increased by using methanol and H₂O at different ratios (CH₂Cl₂:MeOH:H₂O = 9:1:0.1→ 8:2:0.2 and→ 7:3:0.5) to afford 2 fractions. Fr. 1 eluted with (CH₂Cl₂:MeOH = 8:2:0.2) (159 mg) was chromatographed on silica gel column (ϕ 24 × 1.5 cm, 6 g) using gradient elution starting with 100% CH₂Cl₂ and the polarity was increased by using methanol and H₂O at different ratios (CH₂Cl₂:MeOH:H₂O = 9:1:0.1→ 8:2:0.2 and→ 7:3:0.5) to yield 3 subfractions fr. 1-1 to fr. 1-3. Fr. 1-3 (50 mg) was purified on Sephadex LH-20 (ϕ 1.5 × 25 cm, 15 g) eluted with (100% MeOH) to yield compound 7 (20 mg). Compound 7 was obtained as fine white needle crystals, m.p. 234-235°C, *R*_f=0.39 solvent system (CH₂Cl₂:MeOH:H₂O = 8:2:0.2); EI-MS [M]⁺ m/z at 267; ¹HNMR [CD₃OD, 400 MHz] δ: 8.32 (1H, s, H-8), 8.20 (1H, s, H-2), 5.98 (1H, d, *J*=6.4 Hz, Ribose-H-1'), 4.76 (1H, dd, *J*=6.4, 5.2 Hz, Ribose-H-2'), 4.34 (1H, dd, *J*=2.4, 5.2 Hz, Ribose-H-3'), 4.19 (1H, q, *J*=2.8 Hz, Ribose-H-4'), 3.90 (1H, dd, *J*=2.4, 12.4 Hz, Ribose-H-5^a) and 3.76 (1H, dd, *J*=2.4, 12.4 Hz, Ribose-H-5^b); ¹³CNMR [CD₃OD, 100 MHz] δ: 152.1 (C-2), 148.6 (C-4), 119.6 (C-5), 156.2 (C-6), 140.5 (C-8), 89.8 (Ribose-C-1'), 74.0 (Ribose-C-2'), 71.2 (Ribose-C-3'), 86.7 (Ribose-C-4') and 62.0 (Ribose-C-5').

Fr. 2 (288 mg) eluted with (CH₂Cl₂:MeOH:H₂O = 7:3:0.5) was subjected silica gel column (ϕ 28 × 1.5 cm, 11 g) using gradient elution starting with 100% CH₂Cl₂ and the polarity was increased by using methanol and H₂O at different ratios (CH₂Cl₂:MeOH:H₂O = 9:1:0.1→8:2:0.2→7:3:0.5 and→6:4:1) to afford 2 subfractions fr. 2-1 and fr. 2-2. Fr. 2-2 (62 mg) was chromatographed on Sephadex LH-20 (ϕ 1.5 × 25 cm, 15 g) eluted with (100% MeOH) to give compound 8 (15 mg). Compound 8 was obtained as yellowish white amorphous powder, m.p. 289-290 °C, *R*_f= 0.48 solvent system

(CH₂Cl₂:MeOH:H₂O = 7:3:0.5); EI-MS [M]⁺ m/z at 204; ¹HNMR [CD₃OD, 400 MHz] δ: 7.72 (1H, d, *J*=7.6 Hz, H-4), 7.38 (1H, d, *J*=7.6 Hz, H-7), 7.21 (1H, s, H-2), 7.14 (1H, t, *J*=7.6 Hz, H-6), 7.06 (1H, t, *J*=7.6 Hz, H-5), 3.88 (1H, dd, *J*=4, 9.2 Hz, H-11), 3.53 (1H, dd, *J*=3.6, 15.2 Hz, H^b-10) and 3.16 (1H, dd, *J*=9.2, 15.2 Hz, H^a-10); ¹³CNMR [CD₃OD, 100 MHz] δ: 123.7 (C-2), 108.1 (C-3), 117.9 (C-4), 118.6 (C-5), 121.3 (C-6), 111.0 (C-7), 136.9 (C-8), 127.0 (C-9), 27.0 (C-10), 55.2 (C-11) and 173.1 (C-12).

Acid hydrolysis of the isolated glycosides

Complete acid hydrolysis was carried out by refluxing 5 mg of each glycoside with 10 ml of 1.5 N HCl in aqueous methanol for 2 hrs. The hydrolysate was extracted with ethyl acetate, then evaporated to obtain the aglycone. The mother liquor of hydrolysate was neutralized with BaCO₃ to be analyzed by TLC silica gel using solvent system (*n*-PrOH:EtOAc:H₂O = 7:2:1) in the presence of authentic samples⁹.

Biological activities

Total methanol extract of *U. urens* L. aerial parts and their different fractions (petroleum ether, methylene chloride, ethyl acetate and *n*-butanol) were investigated for antioxidant, cytotoxic and anti-hyperglycemic activities.

Antioxidant activity

The antioxidant activity of different concentrations (0.8, 0.4, 0.3,0.2, 0.1 mg/ml) of the methanol extract of *U. urens* L. aerial parts was determined according to the method reported by Kadri *et al* (2011) using the stable radical 2,2- diphenyl-1-picrylhydrazyl (DPPH)¹⁰. Each sample was measured in triplicate by using UV-Vis spectrophotometer at λ_{max}517 nm and the percentage of scavenging was calculated.

Cytotoxic activity

The test was carried out according to the method reported by Skehan *et al.* (1990)¹¹ using 100 µg/ml of the total methanol extract of *U. urens* L. and their different fractions (petroleum ether, methylene chloride, ethyl acetate and *n*-butanol) in DMSO against 4 different cell lines (HepG2, MCF-7, PC3 and HCT) in a single dose experiment (100 µg/ml). The most effective fractions were tested in multiple doses (5, 12.5, 25 and 50 µg/ml) using doxorubicin as a standard. The IC₅₀ was determined.

Anti-hyperglycemic activity

The anti-hyperglycemic activity of the total methanol extract of *U. urens* L. aerial parts was evaluated in STZ-induced diabetic rats according to the method reported by Kumar *et al* (2012)¹².

Biochemical study

Determination of reduced glutathione (GSH):

Reduced glutathione was determined according to the method reported by Beutler (1963)¹³. The values were expressed as (mmoles GSH/L).

Determination of catalase activity (CAT):

Catalase activity was determined according to the procedures reported by Aebi (1984)¹⁴. The activity of the enzyme was expressed in the terms of (U/L).

Determination of superoxide dismutase (SOD):

Superoxide dismutase (SOD) was determined according to the method reported by Nishikimi *et al.* (1972)¹⁵. The activity of the enzyme was expressed in the terms of (U/mL).

Determination of malondialdehyde (MDA):

Malondialdehyde (MDA) concentration was determined according to the method reported by Kei (1978)¹⁶. The activity of the enzyme was expressed in the terms of (nmol/mL).

Determination of serum urea level:

Serum urea level was determined according to the procedures reported by Tobacco *et al.* (1979)¹⁷. Serum urea level was expressed in the terms of (mg/dL).

Determination of serum creatinine level:

Serum creatinine level was determined according to the method reported by Heinegård *et al.* (1973)¹⁸. Serum creatinine level was expressed in the terms of (mg/dL).

Determination of serum ALT and AST level:

Serum ALT and AST level were determined according to the method reported by Bergmeyer *et al.* (1980)¹⁹. Serum ALT and AST level were expressed in terms of (U/L).

Statistical analysis

Comparison between different groups was carried out by one-way analysis of variance test (ANOVA) followed by LSD test. The level of significance was set at $P \leq 0.05$. The Standard Package of Social Sciences (SPSS 11) computer software was used to carry out the statistical analysis.

Histopathological study

Liver slices of the second largest lobe from rats in all groups were excised, immediately fixed in stoppered jars containing 10 % formalin in normal saline routinely processed in ascending grades of alcohol, cleared in xylene then paraffin embedded. Paraffin embedded sections were cut on charged slides (3-5 μ m) and stained with hematoxylin and eosin stain (H & E) to be studied by light microscope²⁰.

Table 1: Effect of methanol extract of *Urtica urens* L. aerial parts on serum GSH, MDA levels, SOD, CAT activities, ALT, AST, Urea and Creatinine levels

Group	Normal control	Diabetic control	Diabetic + glibenclamide	Diabetic + extract
GSH (mmol/L)	1.71 $\pm$ 0.07	0.90 $\pm$ 0.10 ^a	1.19 $\pm$ 0.11 ^a	1.52 $\pm$ 0.04 ^{b,c}
MDA (nmol/mL)	1.29 $\pm$ 0.04	1.90 $\pm$ 0.18 ^a	1.60 $\pm$ 0.10	1.32 $\pm$ 0.09 ^b
SOD (U/mL)	24.88 $\pm$ 1.34	12.31 $\pm$ 0.77 ^a	16.04 $\pm$ 1.02 ^a	20.21 $\pm$ 2.64 ^b
CAT (U/L)	0.22 $\pm$ 0.014	0.16 $\pm$ 0.015 ^a	0.18 $\pm$ 0.007	0.21 $\pm$ 0.008 ^b
Serum ALT (U/L)	22.33 $\pm$ 4.66	39.33 $\pm$ 3.17 ^a	33.33 $\pm$ 4.91	26.33 $\pm$ 5.45 ^b
Serum AST (U/L)	101.66 $\pm$ 3.17	161 $\pm$ 10.44 ^a	134.66 $\pm$ 13.11	114.33 $\pm$ 8.98 ^b
Serum urea (mg/dL)	17.66 $\pm$ 2.60	44.66 $\pm$ 3.48 ^a	39.66 $\pm$ 4.09 ^a	23.66 $\pm$ 4.48 ^{bc}
Serum creatinine (mg/dL)	0.58 $\pm$ 0.07	1.10 $\pm$ 0.102 ^a	0.94 $\pm$ 0.06 ^a	0.76 $\pm$ 0.103 ^b

Data are expressed as mean $\pm$ SEM (n = 7).

Values with different superscripts are significantly different at $P < 0.05$.

^a Significant difference from normal control group ($p < 0.05$).

^b Significant difference from diabetic control group ($p < 0.05$).

^c Significant difference from glibenclamide (+ve control) group ($p < 0.05$).

Figure 1: Compounds isolated from *Urtica urens* L. aerial parts

Figure 2: Free radical scavenging activity of *Urtica urens* L. aerial parts methanol extract at different concentrations

Figure 3: Single dose cytotoxic activity of *Urtica urens* L. aerial parts total methanol extract and different fractions

Figure 4: Multidose cytotoxic activity of *Urtica urens* L. *n*-butanol, ethyl acetate fractions and doxorubicin against PC3 and HEPG2 cell lines
 (a): Multidose cytotoxic activity of *n*-butanol fraction against PC3 cell line.
 (b): Multidose cytotoxic activity of doxorubicin against PC3 cell line.
 (c): Multidose cytotoxic activity of ethyl acetate fraction against HEPG2 cell line.
 (d): Multidose cytotoxic activity of doxorubicin against HEPG2 cell line.

Figure 5: Effect of methanol extract of *Urtica urens* L. aerial parts on serum glucose level in rats with STZ induced hyperglycemia

a: Significant difference from normal control group ($p < 0.05$).
 b: Significant difference from diabetic control group ($p < 0.05$).
 c: Significant difference from glibenclamide (+ve control) group ($p < 0.05$).

Figure 6: Light micrographs of haematoxylin and eosin (H and E $\times 400$) staining showing:
 Photomicrograph (A): Liver sections from normal control rats.
 Photomicrograph (B): Liver sections from diabetic control rats.
 Photomicrograph (C): Liver sections from diabetic rats receiving glibenclamide drug.
 Photomicrograph (D): Liver sections from diabetic rats receiving *Urtica urens* L. aerial parts methanol extract

RESULTS AND DISCUSSION

Phytochemical investigation

GC-MS analysis of *Urtica urens* L., fatty acid methyl esters

The total percentage of saturated fatty acids in *Urtica urens* L. aerial parts was (24.48%), which is higher than the total percentage of unsaturated fatty acids (20.80%). The main saturated fatty acids were hexadecanoic acid methyl ester (17.90%) and methyl stearate (2.43%), while the main unsaturated fatty acids were 9,12-Octadecadienoic acid methyl ester (8.93%) and 9,12,15-Octadecatrienoic acid methyl ester (6.97%).

Phytochemical investigation of *U. urens* L. aerial parts methanol extract led to isolation of eight compounds (Figure 1). Structure elucidation of the isolated pure compounds was assisted using different spectroscopic techniques e.g. UV, IR, EI-MS, ^1H NMR and ^{13}C NMR.

Compound **1** and **3** gave positive Liebermann's and Salkowski's tests, which suggested the sterol nature of these compounds. Based on IR, ^1H NMR and ^{13}C NMR, m.p., and Co-TLC with authentic β -sitosterol and β -sitosterol-3-*O*- β -D-glucoside, it was concluded that compound **1** is 3- β -stigmast-5-en-3-ol (β -sitosterol) and compound **3** is β -sitosterol-3-*O*- β -D-glucoside. This was confirmed by comparing the obtained data to those published in literature^{21,22}.

Compound **2** was obtained as yellow crystalline solid. EI-MS of the compound showed molecular ion peak $[\text{M}]^+$ at m/z 192. The results of UV spectroscopy revealed that compound **2** has λ_{max} at 228, 298, 345 nm. Bathochromic shift was observed in addition of NaOH and NaOAc shift reagents to methanol solution of compound **2**, which confirm the presence of free 7-hydroxyl group. However, no effect was observed by the addition of AlCl_3 ,

AlCl_3/HCl and NaOAc/boric acid, which indicates the absence of the ortho-di hydroxy group. The ^1H NMR spectrum, displayed signals characteristics of a 6,7-dioxygenated coumarin. Presence of two doublets at δ 6.19 (1H, d, $J=9.6$ Hz) and δ 7.85 (1H, d, $J=9.6$ Hz) characteristic of H-3 and H-4 protons respectively of the pyrone ring of a coumarin. The presence of two aromatic proton singlets at δ 7.20 and δ 6.81 were assigned to H-5 and H-8 respectively. A singlet methoxy group attached to C-6 at δ 3.91. The ^{13}C NMR spectrum of compound **2**, revealed the presence of ten carbons including one methoxy, four methines and five quaternary carbons. A peak at δ 160.4 was assigned to the carbonyl function (C-2) of coumarin derivatives while the signal at δ 150.9 revealed the presence of a hydroxyl functional group at the C-7 position. A signal at δ 55.8 confirmed the presence of methoxy carbon at the C-6 position. Other signals at, δ 112.0, 143.7, 109.0, 145.0, 102.8, 150.2 and 111.1 were attributed to C-3, C-4, C-5, C-6, C-8, C-9 and C-10, respectively. From the above-mentioned data, m. p., UV, EI-MS, ^1H NMR and ^{13}C NMR data, compound **2** was identified as 7-hydroxy-6-methoxycoumarin (7-Hydroxy-6-methoxy-2H-1-benzopyran-2-one) or scopoletin. This was confirmed by comparing the obtained data to those published in literature^{23,24}.

Compound **4** was obtained as yellow amorphous powder. The UV pattern showed that, it is flavonol. ^1H NMR spectrum of compound **4**, showed meta coupled doublets at δ 6.07, 6.28 ($J=1.6$ Hz), assigned to H-6 and H-8, respectively confirming 5,7 dihydroxylation, while 3',4'-dihydroxylation was confirmed by two doublets and one doublet of doublet signals at δ 7.63, 6.78, and 7.53 each integrated for one proton with ($J=1.6$, 8.8 Hz) and ($J=1.6$, 8.4 Hz) for meta and ortho coupling, these proton signals were assigned to H-2', H-5' and H-6', respectively. The ^{13}C NMR spectrum of compound **4** showed 15 signals including carbonyl signal at δ 175.9 (C-4), seven oxygenated quaternary carbons (δ 146.5, 135.8, 161.0, 164.1, 156.8, 144.8, and 147.3), two sp^2 quaternary carbons (δ 103.1 and 122.7) and five sp^2 tertiary

carbons (δ 97.8, 93.0, 114.5, 114.8 and 120.2). Comparing compound **4** with authentic sample using co-chromatography, m.p., UV, ^1H NMR and ^{13}C NMR with those reported in literature^{25,26}, revealed that compound **4** is 3,5,7,3',4'-pentahydroxy flavones (quercetin).

Compound **5** was obtained as colorless needle shaped crystals. By comparing m.p. and IR spectral data with those reported in literature^{27,28}, and Co-TLC with authentic sample, compound **5** was identified as succinic acid.

Compound **6** was obtained as pale yellow shiny needle crystals, UV spectral data of compound **6** with different shift reagents, indicated the presence of free hydroxyl groups at 5 and 4' positions (bathochromic shifts with AlCl_3 and NaOH , respectively). No bathochromic shift was observed with NaOAc which confirms substitution at position 7. No orthodihydroxyl system was expected to be present due to lack of shift with NaOAc /Boric acid. However, the 3-*O*-substituted flavanol structure was anticipated from the location of band I and II at 340 nm and 266 nm (in methanol), respectively. Therefore, the UV results suggest 3,7-*O*-di substituted flavanol glycoside. ^1H NMR spectral data of compound **6**, showed two meta coupled protons resonating at δ 6.49, 6.75 ($J=2$ Hz), assigned to H-6 and H-8, respectively. Furthermore, ring B protons were observed at δ 6.96 and 7.82 ($J=8.8$ Hz) as ortho coupled doublets due to the free rotation of the phenyl ring, each signal integrated for 2 protons and were assigned to H-3', H-5' and H-2', H-6', respectively. The ^1H NMR spectral data of compound **6** also showed two doublets, at δ 0.96 ($J=5.6$ Hz) and 1.28 ($J=6$ Hz), indicated the presence of two sets of rhamnose methyl protons and the signals for the anomeric protons appeared as doublets at δ 5.58 at δ ($J=1.6$ Hz) and 5.42 ($J=1.6$ Hz) respectively. The ^{13}C NMR spectrum of compound **6**, showed 15 signals that could be attributed to the aglycone and 12 signals for two sugar moieties. In ^{13}C NMR spectrum of compound **6**, signals at δ 135.0 and 162.1 were assigned for C-3 and C-7. The other evidence for the glycosylation of 3,7 hydroxyl groups was its effect on C-3, C-7 signal by an upfield shift by $\Delta\delta$ 0.2 and 1.6 respectively when compared with aglycone; this upfield shift was accompanied with downfield shift for neighboring carbons signals of C-2, C-4, C-6 and C-8 at δ 158.4, 178.4, 98.4 and 94.2 respectively. Signals at δ 115.1, 130.5 were assigned to oxygen free aromatic carbons (C-3', C-5') and (C-2', C-6') respectively. Signals at δ 121.0, 160.3 were assigned to (C-1', C-4') respectively of the B-ring of a flavanol structure. Other signals at δ (161.6, 156.7 and 106.1) were assigned to C-5, C-9 and C-10 respectively of the A-ring of a flavanol structure. Two anomeric carbon signals were observed at δ 99.1 and 102.1. The remaining sugar carbons appearing at δ 16.2-72.2 were characterized from NMR data. The result of acid hydrolysis afforded rhamnose sugar and an aglycone identical as kaempferol (Co-TLC) comparing with authentic sample. The supposed structure of compound **6** was kaempferol 3,7-*O*- α -L-dirhamnoside (kaempferitrin). The α -configuration of two rhamnose moieties were concluded from the signals of anomeric protons resonating at δ 5.42 and 5.58 ($J=1.6$ Hz) which were downfield. Also, the α -linkage of two rhamnose moieties to hydroxyl group at positions 3 and 7 was evident from the small coupling constant ($J=1.6$ Hz) of (3-*O*-rha H-1 and 7-*O*-rha H-1'). By comparing m.p., UV, ^1H NMR and ^{13}C NMR data with the previously published data^{29,30}, compound **6** was identified as kaempferol 3,7-*O*- α -L-dirhamnoside (Kaempferitrin). This is the first time to be isolated from *U. urens* L. aerial parts.

Compound **7** was obtained as fine white needles crystal. EI-MS of the compound showed an odd number molecular ion peak $[\text{M}]^+$ at m/z 267. The odd number of molecular weight 267 indicated the presence of odd number of nitrogen in this

compound according to nitrogen rule. ^1H NMR spectrum of compound **7**, showed two singlets at δ 8.20 and 8.32 characteristic of two vinyl hydrogens of purine group (H-2 and H-8 respectively). Three methine protons at δ 4.76 (1H, dd, $J=6.4$, 5.2 Hz), 4.34 (1H, dd, $J=2.4$, 5.2 Hz) and 4.19 (1H, q, $J=2.8$ Hz) characteristic of H-2', H-3' and H-4' of sugar part respectively. Signals of ABX-type coupled hydroxymethyl protons at δ 3.76 (1H, dd, $J=2.4$, 12.4 Hz) and 3.90 (1H, dd, $J=2.4$, 12.4 Hz) characteristic of H-5'^a and H-5'^b respectively. Signal of the anomeric proton at δ 5.98 (1H, d, $J=6.4$ Hz) characteristic of H-1' of sugar part. The large coupling constant of H-1' ($J=6.4$ Hz) of ribose sugar suggesting the β -linkage of D-ribose moiety to nitrogen atom at positions 9 and the α configuration of H-1' of ribose moiety. ^{13}C NMR spectrum showed ten carbon signals (five carbon signals corresponding to sugar part and another five carbon signals corresponding to adenine group). The anomeric carbon of the sugar moiety (C-1') appeared at δ 89.8. The other carbons signals of sugar moiety were found at δ 62.0, 71.2, 74.0 and 86.7 and were assigned to (C-5', C-3', C-2' and C-4') respectively. These were in agreement with those of β -D-ribose. Signal at δ 156.2 was assigned to (C-6) of adenine group and appeared down fielded due to amino group at (C-6). The other carbon signals of adenine group were found at δ 152.1, 148.6, 119.6 and 140.5 were assigned to (C-2, C-4, C-5 and C-8) respectively. By comparing m.p., EI-MS, ^1H NMR and ^{13}C NMR data with previously published data^{31,32} and direct Co-TLC with authentic sample, compound **7** was identified as 6-amino-9- β -D-ribofuranosyl-9H-purine (β -adenosine). This compound is the first time to be isolated from *U. urens* L. aerial parts.

Compound **8** was obtained as yellowish white amorphous powder, EI-MS fragmentation pattern of the compound showed molecular ion peak at m/z 204 $[\text{M}]^+$. ^1H NMR spectrum indicated the presence of four aromatic hydrogen atoms (2 doublets at δ 7.38, 7.72 and 2 triplets at δ 7.06, 7.14) having similar coupling constants ($J=7.6$ Hz). The remaining singlet at δ 7.21 corresponds to H-2 of the indole ring, which is indicative of a 3-substituted indole structure, as found in tryptophan. Also, an ABX system was present at δ 3.88 (1H, dd, $J=4$, 9.2 Hz) which could be attributed to the alpha proton, at δ 3.16 (1H, dd, $J=9.2$, 15.2 Hz) and 3.53 (1H, dd, $J=3.6$, 15.2 Hz) which could be attributed to beta protons of this amino acid. ^{13}C NMR spectrum of compound **8** revealed the presence of eleven carbon signals, including eight carbon signals attributed to a 3-substituted indole skeleton at δ (123.7, 108.1, 117.9, 118.6, 121.3, 111.0, 136.9 and 127.0) which are corresponding to (C-2, C-3, C-4, C-5, C-6, C-7, C-8 and C-9 respectively). In addition to the signals attributable to the 3-substituted indole skeleton, ^{13}C NMR spectra also showed signals for a methylene carbon at δ 27.0 which corresponds to (C-10), methine carbon signal at δ 55.2 which corresponds to (C-11) and carbonyl carbon signal at δ 173.1 which corresponds to (C-12). By comparing m.p., EI-MS, ^1H NMR and ^{13}C NMR data with previously published data^{33,34}, compound **8** was identified as 2-amino-3-(1H-indol-3-yl) propanoic acid (tryptophan). This is the first time to be isolated from *U. urens* L. aerial parts.

Biological investigation

Antioxidant activity

U. urens L. aerial parts methanol extract showed a dose dependent antioxidant activity (Figure 2). These results aligned with the reported literature⁷ and supported recent studies on unrevealed anticancer effect of *U. urens* L.

Cytotoxic activity

The present study is the first report evaluating the cytotoxic activity of *U. urens* L. total extract and different fractions against, liver carcinoma (HEPG2), prostate carcinoma (PC3), breast carcinoma (MCF-7) and colon carcinoma (HCT) cell lines. The results of single dose experiment indicated that the plant methanol extract has significant cytotoxic activity against HEPG2 cell line compared to the other cell lines. Ethyl acetate and *n*-butanol fractions showed the highest cytotoxic activity against HEPG2 and PC3 cell lines with percent inhibition 74% and 87% respectively (Figure 3). Further investigations were performed as multiple doses test on *n*-butanol, ethyl acetate fractions against PC3 and HEPG2 cell lines, using doxorubicin as positive control. In comparison with doxorubicin it was found that ethyl acetate (IC₅₀=2.9 µg/ml) was more potent than doxorubicin against HEPG2 cell line, while *n*-butanol fraction presented less activity (IC₅₀=35.8 µg/ml) against PC3 cell line (Figure 4). These results may suggest the use of *U. urens* L. as powerful anticancer agent.

Anti-hyperglycemic activity

The administration of *U. urens* L. aerial parts methanol extract to STZ-induced diabetic rats significantly reduced serum glucose levels in comparison with diabetic control and glibenclamide groups (Figure 5). These results may reflect the antioxidant activity of *U. urens* L. aerial parts via its flavonoidal and phenolic contents.

Biochemical study

The biochemical study carried out to evaluate the effect of *U. urens* L. total methanol extract on oxidative stress, hepatic and renal damage associated secondary complications in STZ-induced diabetic rats. Several biomarkers were involved as serum catalase (CAT), superoxide dismutase (SOD) enzymatic activities, glutathione (GSH) level, malondialdehyde (MDA) content; serum ALT, AST, creatinine and urea levels. The treatment of diabetic rats with total methanol extract of *U. urens* L. aerial parts significantly ($p < 0.05$) raised the antioxidant GSH level, SOD and CAT enzyme activities but decreased MDA level (Table 1). These results high lightened the remarkable role of *U. urens* L. in regaining balance between the radical scavenging and radical generating systems at STZ-induced diabetic rats via decreasing the glycolytic deactivation of enzymatic and non-enzymatic antioxidants. *U. urens* L. total extract is even more potent than glibenclamide treated diabetic group. Administration of 250 mg/kg by weight of *U. urens* L. aerial parts extract significantly reduced the serum levels of AST, ALT, urea and creatinine (Table 1) with respect to diabetic control rats. Such reduction was comparable to the basal normal levels of these biomarkers.

Histopathological study

Histopathological investigation of the excised liver lent further confirmation to the effect of *U. urens* L. aerial parts methanol extract on diabetic rats as shown in (Figure 6). Liver sections of normal control rats have typically arranged hepatocytes in a single cell plate radiating from the central vein. The hepatocytes appear polygonal in shape, joined to one another in anastomosing plates. Whether liver sections from diabetic control rats showed a congested and dilated portal vein with portal and periportal inflammatory cells infiltration. The inflammatory cells (neutrophils and plasma cells) invade the necrotic peripheral limiting plate of hepatocytes surrounding the portal triad (piecemeal necrosis). Liver sections from diabetic control rats receiving glibenclamide showed normally arranged hepatocytes

with vacuolated cytoplasm, dilated congested portal vein, little periportal inflammatory cells infiltration and ductal proliferation. Liver sections of rats treated with 250 mg/kg b.w. of *Urtica urens* L. herb extract showed normally arranged hepatocytes radiating from central vein with little inflammatory cells infiltration and fibroblast are also seen. These results revealed that *U. urens* L. aerial parts methanol extract possessed hepatoprotective effect in diabetic rats. These results were confirmative to those reported on hepatoprotective effect of *U. urens* L. seed extract on CCl₄ dependent hepatotoxic rats⁸. These biochemical and histopathological studies of *U. urens* L. total extract effect is first reported.

ACKNOWLEDGEMENT

The authors would like to express their gratitude to Associate Prof. Darine A. Mohamed, Associate Professor of Pathology, Faculty of Medicine, Tanta University; for her devoted help in carrying out the histopathological study.

REFERENCES

1. Boulos L. Flora of Egypt. 1st ed. Cairo, Egypt: Al Hadara publishing; 1999. p. 373.
2. Parish S. The immigrant plants of southern California. Bull South Calif Acad Sci. 1920; 19:3–30.
3. Shaltout KH, Sharaf El- din A and Ahmed DA. Plant life in the Nile delta. Egypt; 2010.
4. Kavtaradze NS. Phenolic compounds from *Urtica urens* growing in Georgia. Chem Nat Compd. 2003;39(3):314.
5. Patten G. Medicinal plant review *Urtica*. Aust J Med Herbal. 1993; 5:5–13.
6. Marrasini C, Acevedo C, Miño J, Ferraro G, Gorzalczyński S. Evaluation of Antinociceptive, Antiinflammatory Activities and Phytochemical Analysis of Aerial Parts of *Urtica urens* L. Phyther Res. 2010;24(March):1807–12.
7. Jimoh F, Adedapo A, Aliero A, Afolayan A. Polyphenolic and biological activities of leaves extracts of *Argemone subfusiformis* (Papaveraceae) and *Urtica urens* (Urticaceae). Int J Trop Biol Conserv. 2010;58(December):1517–31.
8. Alaattin Sen, Barbaros Sahin, Hizlan H. Agus, Merve Bayav, Sevim H and Semiz A. Prevention of Carbon Tetrachloride-Induced Hepatotoxicity by *Urtica urens* in Rats. J Appl Biol Sci. 2007;1(3):29–32.
9. Harborne JB, Mabry TJ and Mabry H. The Flavonoids. New York, San Francisco: Academic press; 1975. p. 1-44.
10. Kadri A, Gharsallah N, Gdoura R, Chobba I B, Zarai Z, Bekir A and Damak M. Chemical constituents and antioxidant activity of the essential oil from aerial parts of *Artemisia herba-alba* grown in Tunisian semi-arid region. African J Biotechnol. 2011;10(15):2923–9.
11. Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren J T, Bokesch H, Kenney S and Boyd MR. New colorimetric cytotoxicity assay for anticancer-drug screening. J Natl Cancer Inst. 1990;82(13):1107–12.
12. Kumar S, Kumar V and Prakash OM. Antidiabetic and hypolipidemic activities of *Kigelia pinnata* flowers extract in streptozotocin induced diabetic rats. Asian Pac J Trop Biomed. 2012; 2:543–6.
13. Beutler E. Improved method for the determination of blood glutathione. J Lab Clin Med. 1963; 61:882–8.
14. Aebi H. Catalase in vitro. Methods Enzymol. 1984; 105:121–6.
15. Nishikimi M, Roa NA and Yogi K. Measurement of superoxide dismutase. Biochem Biophys Res Commun. 1972; 46:849–54.

16. Kei S. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clin Chim acta. 1978;90(1):37–43.
17. Tobacco A, Meattini F, ME and et al. Simplified enzymic/colorimetric serum urea nitrogen determination. Clin Chem. 1979; 25:336–7.
18. Heinegard D and Tiderstrom G. Determination of serum creatinine by a direct colorimetric method. Clin Chim Acta. 1973;43(3):305–10.
19. Bergmeyer HU and et al. IFCC methods for measurement of catalytic concentrations of enzymes. Clin Chim Acta. 1980; 105:147–72.
20. Bancroft JD and Stevens A. Histopathological Stains and Their Diagnostic Uses. Churchill Livingstone, editor. Edinburgh London and New york: Churchill Livingstone; 1975. p.29.
21. Al-Shammari L A, Hassan W H and Al Youssef HM. Phytochemical and biological studies of *Carduus pycnocephalus* L. J Saudi Chem Soc [Internet]. King Saud University; 2015 [cited 2016 Oct 2];19(4):410–6. Available from: <http://dx.doi.org/10.1016/j.jscs.2012.05.002>
22. Viswanadh GS, Atchuta Ramaiah P, Laatsch H and Maskey R. Chemical constituents of the heartwood and barks of *Homonoia riparia*. J Trop Med Plants. 2006;7(2):267–73.
23. Silva WPK, Deraniyagala SA, Wijesundera RLC, Karunanayake EH and Priyanka UMS. Isolation of scopoletin from leaves of *Hevea brasiliensis* and the effect of scopoletin on pathogens of *H. brasiliensis*. Mycopathologia. 2002;153(4):199–202.
24. Prabowo WC, Wirasutisna KR and Insanu M. Isolation and Characterization Of 3-Acetyl Aleuritic Acid and Scopoletin From Stem Bark of *Aleurites Moluccana* (L.) Willd. Int J Pharm Sci. 2013;5(3):851–3.
25. Augustine AA and Ufuoma O. Flavonoids from the leaves of *Physalis angulata* Linn. African J Pharm Res Dev [Internet]. 2013 [cited 2016 Oct 5];5(1):40–3. Available from: <Go to ISI>://WOS:000209339700415
26. Fatma A A, Inas MK and Samar YD. Flavonoids of *Neoturularia aculeolata* plant. J Pharm Nutr Sci. 2011; 1:134–9.
27. Premkumar T, Selvakumar R, Rath NP and Govindarajan S. Synthesis and Spectroscopic, Thermal and Crystal Structure Studies of Hydrazinium Hydrogensuccinate. South African J Chem. 2014; 67:85–90.
28. Mitra T, Sailakshmi G, Gnanamani A and Mandal AB. Studies on Cross-linking of succinic acid with chitosan/collagen. Mater Res [Internet]. 2013 [cited 2016 Oct 7];16(4):755–65. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1516-14392013000400009&lng=en&nrm=iso&tng=en
29. Toker G, Memişoğlu M, Yeşilada E. and Aslan M. Main flavonoids of *Tilia argentea* DESF. ex DC. leaves. Turkish J Chem. 2004;28(6):745–9.
30. Yang S, Liu M, Liang N, Zhao Q, Zhang Y, Xue W and Yang S. Discovery and antitumor activities of constituents from *Cyrtomium fortunei* (J.) Smith rhizomes. Chem Cent J [Internet]. 2013 [cited 2016 Nov 14];7(1):24. Available from: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3574041&tool=pmcentrez&rendertype=abstract>
31. Domondon DL, He W, De Kimpe N, Ho M and Poppe J. b-Adenosine, a bioactive compound in grass chaff stimulating mushroom production. Phytochemistry. 2004; 65:181–7.
32. Ciuffreda P, Casati S and Manzocchi A. Complete ¹H and ¹³C NMR spectral assignment of α- and β-adenosine, 2'-deoxyadenosine and their acetate derivatives. Magn Reson Chem. 2007;45(July):781–4.
33. Kotanen S, Huybrechts J, Cerstiaens A, Zoltan K, Daloze D, Baggerman G, Forgo P, De Loof A and Schoofs L. Identification of tryptophan and b-carboline as paralytins in larvae of the yellow mealworm, *Tenebrio molitor*. Biochem Biophys Res Commun. 2003; 310:64–71.
34. Wang SL, Li HT, Zhang LJ, Lin ZH and Kuo YH. Conversion of Squid Pen to Homogentisic Acid via *Paenibacillus* sp. TKU036 and the Antioxidant and Antiinflammatory activities of homogentisic acid. Mar Drugs. 1999;14(10):183.

Cite this article as:

Hosam M. El-Seadawy et al. Phytochemical and biological investigation of *Urtica urens* L. growing in Egypt. Int. Res. J. Pharm. 2018;9(1):25-35 <http://dx.doi.org/10.7897/2230-8407.0915>

Source of support: Nil, Conflict of interest: None Declared

Disclaimer: IRJP is solely owned by Moksha Publishing House - A non-profit publishing house, dedicated to publish quality research, while every effort has been taken to verify the accuracy of the content published in our Journal. IRJP cannot accept any responsibility or liability for the site content and articles published. The views expressed in articles by our contributing authors are not necessarily those of IRJP editor or editorial board members.