**Supplementary materials**

**LC/ESI-MS/MS profiling of *Ulmus parvifolia* extract and evaluation of its anti-inflammatory, cytotoxic and antioxidant activities**

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**Experimental**

***Plant material***

Leaves and branches of *Ulmus parvifolia* (Ulmaceae) were collected from EL-Orman Public Garden, Giza, Egypt in Mars 2012. The plant was identified by Dr. Threse Labib head specialist of plant identification in El-Orman Garden. A voucher specimen (Reg. No. U.P.12) has been deposited in the herbarium of Pharmacognosy Department, Faculty of Pharmacy, Helwan University, Cairo, Egypt.

***Fractionation:***

The dried powder of *U. parvifolia* leaves and branches (100g) was extracted with 80% methanol (6x250 ml) at 55 ͦ C and the methanolic extract was concentrated under reduced pressure at 45 ͦ C to yield a brown residue (8.5 g). The residue was defatted with n-hexane (4 x 100 ml) at 45 ͦ C and the defatted residue (5.9 g) was suspended in water (100ml) and partitioned against chloroform (4x100ml), ethylacetate (5x100ml) and finally with n- butanol (5x100 ml). The obtained fractions (1.9 g, 282 mg and 457 mg respectively) were analysed for their constituents on LC- mass instrument.

***Isolation and purification of compound 5:***

The dried defatted methanolic extract (170 gm) of *U. parvifolia* stem and branches was chromatographed on a porous polymer gel Mitsubishi Diaion HP-20 column. Elution was performed using H2O, 25 %, 50%, 75% H2O: MeOH and finally 100 % methanol. The fractions eluted with 50%, 75% and 100 % MeOH were combined after TLC analysis (BuOH-EtOH-NH4OH 7:2:5). The combined fraction (25 g) was subjected to silica gel VLC using CH2Cl2 followed by CH2Cl2-MeOH . Five fractions (I- V) were obtained after monitoring on TLC. Fraction III eluted at CH2Cl2:MeOH (85:15– 55:45) (14 g) was subjected to repeated silica gel column chromatography and sephadex LH 20 then purified on PTLC ( BuOH-MeOAc-H2O 4:1:0.5) to yield compound **5** (19 mg).

***LC/MS analysis:***

LC/MSanalysiswas performed on triple stage quadrupole mass spectrometer, TSQ Quantum Access MAX, Thermo Scientific, New York, USA, equipped with an electrospray ionization (ESI) operated in the positive ionization mode (60 ev). Chromatography was carried on Accela U-HPLC system which was composed of Accela 1250 quaternary pump and Accela open autosampler, New York, USA (operated at 25oC). Hypersil Gold column (C18 bonded ultrapure silica based column) 50 mm × 2.0 mm (1.9 μm), Thermoscientific, New York, USA was employed. Isocratic elution was carried out using freshly prepared Acetonitrile (A): 0.2% formic acid mixture (B) (90:10, v: v) at flow rate 250 μl/min. X-calibur software version 2.2 was used to control all parameters of UPLC , MS and analysis of the obtained data.

***Biological Material***

Raw murine macrophage (RAW 264.7), human hepatocarcinoma cell line (HepG2), human breast adenocarcinoma cell line (MCF-7), human colorectal carcinoma cells (HCT-116) andhuman colon adenocarcinoma cell line (CACO-2) were provided by the American Type Culture collections from Cambrex, BioScience (Copenhagen, Denmark). L-glutamine, penicillin G sodium, streptomycin sulphate, amphotericin B, 3 -( 4, 5 – dimethylthiazol – 2 – yl ) – 2 , 5 –diphenyl-tetrazoliumbromide (MTT), isopropanol, LPS (lipopolysaccharide), Dexamethasone, DPPH, ascorbic acid and DMSO were purchased from Sigma/Aldrich, (USA).

***Anti-inflammatory activity (NO inhibition method)***

Raw murine macrophages (RAW 264.7) were seeded in 96-well plates at 0.5×105 cells / well for 2 hours in RPMI without phenol red. The cells were stimulated with LPS with final concentrations of 100 µgmL**−1**. Stimulated cells after two extra hours were either treated with 100 µg/ml of the test samples and dexamethasone (50 µg/ml) as a potent anti-inflammatory agent, left with the LPS alone or left untreated at all as a negative control. After total 24 hours' time interval the supernatants were removed and assessed for Nitric oxide (NO)*.* In each well of a flat bottom 96 well- microplate, 40 µl freshly prepared Griess reagent was mixed with 40 µl cell supernatant of different concentrations of sodium nitrite (12.5, 20, 50, 100 µg/ml). The plate was incubated for 10 min in the dark and the absorbance of the mixture at 540 nm was determined using the microplate ELISA reader.A standard curve relating NO concentration in µmol/L to the absorbance is constructed, from which the NO level in the cell supernatant is computed by interpolation.The NO level of each of the tested cell supernatant was expressed as NO level of the tested cell supernatant x100/ NO level of the control (Green *et al*., 1982).

***Cytotoxic activity (MTT assay):***

Cytotoxicity conducted by MTT assay was adapted according to Mosmann (1983). The cells (0.5X105 cells/ well) were cultured in RPMI 1640 supplemented with 10% heat inactivated fetal bovine serum (FBS) and 1% penicillin/streptomycin and maintained at 37°C in an atmosphere of 5% CO2. The cells were placed in a flat bottom TC 96-well micro plate, and treated with 20 µl of different concentrations of the test samples (6.125, 12.5, 25, 50, 100 μg/ ml) for 24 hrs. After incubation, media were removed and 40 µl MTT solution per well were added and incubated for an additional 4 h. MTT crystals were solubilized by adding 180 µl of acidified isopropanol per well and each plate was shacked at room temperature, followed by photometric determination of the absorbance at 570 nm using micro-plate ELISA reader [29]. Three times repeats were performed for each concentration and the average was calculated. Data were expressed as the percentage of relative viability compared with the untreated cells in DMSO. The cytotoxicity indicated by <100% relative viability. Percentage of relative viability was calculated using the following equation:

[Absorbance of treated cells/ Absorbance of control cells)] X 100

The concentration of extracts required to kill 50% of cell population (IC50) was determined from data generated by plotting a dose-response curve

***Antioxidant activity (DPPH assay):***

The ability of extract to scavenge free radicals was assayed according to (Ratty et al., 1988). In a flat bottom 96 well-microplate, a total test volume of 200 µl was used. In each well, 20µl of different concentrations (12.5, 25, 50, 100 µg/ml) of test samples were mixed with 180µl of ethanolic DPPH and incubated for 30 min at 37ºC.Triplicate wells were prepared for each concentration and the average was calculated. Then photometric determination of absorbance at 520 nm was performed by microplate ELISA reader.The half maximal scavenging capacity (SC50) values for each test sample and ascorbic acid used as positive control was estimated via dose-response curve. SC50 of each sample was calculated using the curve equation.