Supplementary Information

Characterization of Compounds:

Desgalactotigonin- is an amorphous solid, mp 235-237 ºC; Q-TOF Mass (m/z) 1057 $[M + Na]^+$; ^{1}H NMR (Pyridine-d_5, 600 MHz) δ 0.51 (1H, m), 0.64 (3H, s), 0.70 (3H, d, J = 4.8 Hz), 0.82 (4H, s), 0.89 (1H, m), 1.05 (3H, m), 1.15 (5H, m), 1.38 (4H, m), 1.61 (8H, m), 1.79 (2H, m), 1.99 (2H, m), 3.61 (2H, m), 3.92 (5H, m), 4.08 (6H, m), 4.19 (1H, m), 4.24 (3H, m), 4.43 (3H, m), 4.58 (3H, m), 4.89 (1H, d, J = 7.2 Hz), 5.20 (1H, d, J = 8.4 Hz), 5.25 (1H, d, J = 7.8 Hz), 5.43 (1H, s), 5.59 (1H, d, J = 7.2 Hz); ^{13}C NMR [Pyridine-d_5, 150 MHz] δ 12.5 (CH_3), 15.3 (CH_3), 16.8 (CH_3), 17.7 (CH_3), 21.5 (CH_2), 29.1 (CH_2), 29.5 (CH_2), 30.1(CH_2), 30.8 (CH), 32.0 (CH_2), 32.4 (CH_2), 32.6 (CH_2), 35.0 (CH_2), 35.5 (CH), 36.0 (C), 37.4 (CH_2), 40.4 (CH_2), 41.0 (C), 42.2 (CH), 44.9 (CH), 54.6 (CH), 56.7 (CH), 60.5 (CH_2), 62.7 (CH_2), 63.2 (CH), 63.2 (CH_2), 67.1 (CH_2), 67.6 (CH_2), 70.7 (CH), 70.9 (CH), 71.2 (CH), 73.4 (CH), 75.3 (CH), 75.6 (CH), 75.8 (CH), 76.5 (CH), 77.6 (CH), 77.9 (CH), 78.0 (CH), 78.9 (CH), 79.0 (CH), 80.2 (CH), 81.4 (CH), 81.6 (CH), 86.9 (CH), 102.6 (CH), 105.1 (CH), 105.2 (CH), 105.4 (CH), 109.5 (C) (Fig-1a) .

Oleanolic acid 3-O-β-D-glucuronide- is an amorphous solid, mp 256-258 ºC; Negative ESI-MS, m/z (%): 631.7([M-H], 100), 455.6 ([M-H]−176.22 for loss of glucuronic acid moiety). ^{1}H NMR OAG (Pyridine-d_5, 600 MHz) δ 0.83 (5H, m, 1-CH_3), 1.01 (12H, m, 4-CH_3), 1.22 (2H, m), 1.33 (10H, m, 2-CH_3), 1.50 (4H, m), 1.66 (1H, m), 1.95 (7H, m), 2.22 (3H, m), 3.34 (2H, m), 4.10 (1H, s), 4.30 (1H, s), 4.49 (2H, m), 4.95 (1H, s), 4.87 (1H, s), 5.48 (2H, s); ^{13}C NMR [Pyridine-d_5, 150 MHz] δ 15.0 (CH_3), 16.2 (CH_3), 17.8 (CH_3), 18.1 (CH_3), 19.2 (CH_2), 24.4 (CH_2), 24.5 (CH_2), 27.0 (CH_2), 27.2 (CH_2), 29.0 (CH_2), 29.1 (CH_2), 30.7 (CH_2), 31.7 (CH_2), 34.0 (CH_2), 34.1 (CH_2), 35.0 (CH), 37.7 (C), 39.4 (C), 40.2 (CH_2), 40.5 (CH_2), 42.7 (CH_2), 42.9 (C), 47.2 (CH), 47.4 (CH), 48.7 (CH), 56.6 (CH), 74.2 (CH), 76.2 (CH), 77.9 (CH), 79.0 (CH), 80.6 (CH), 89.9 (CH), 107.6 (CH), 123.3 (CH), 145.6 (C), 181.0 (C) (Fig-1b).

Materials and Methods

Plant material

Matured fruits of *Chenopodium album* (Linn) were collected from the medicinal plant garden of R. K. Mission, Narendrapur, Kolkata on April, 2012. The plant is not an endangered or protected species. It was authenticated by Dr. Debjani Basu, Asst. Director, Botanical Survey of India, Howrah (West Bengal, India). A voucher specimen

(No.786) was deposited in the Chemistry Department, Indian Institute of Chemical Biology. The black seeds were segregated from the pericarp of the fruits and then ground in an industrial blender.

Preparation of extract

Powdered seeds (3 Kg) were defatted by successive percolation with petroleum ether (60-80°C) and chloroform, then extracted by percolation with methanol. The solvent was distilled off under reduced pressure (<50 °C) using a rotary evaporator (Eyela, Tokyo, Japan). Removal of inorganic salts from the methanol extract was effected by partitioning between *n*-butanol and water. The *n*-butanol part was concentrated by reduced pressure distillation to yield 35g of greenish brown mass.

Purification of extract

The greenish brown mass obtained from the *n*-butanolic portion was passed through silica gel (60-120) column and eluted with solvents in an increasing order of polarity starting with chloroform and ending with methanol. The fractions were further purified through repeated column chromatography in conjunction with thin layer chromatography. This ultimately yielded two pure products, the major one with eluent $CHCl_3$: MeOH (75:25) and the minor one with eluent $CHCl_3$: MeOH (65:35); the rest were inseparable mixtures. The major product (yield 0.004% on dry weight basis of the plant material), which was crystallized from MeOH and characterized as desgalactotigonin via spectroscopic analysis, viz. mass, ^{13}C NMR, and ^{1}H NMR spectroscopy followed by comparison with data reported in the literature (Yan et al., 1996). This is the first report of its isolation from *Chenopodium album*. The other product was characterized as oleanolic acid 3- *O*-*β*-D-glucoronide (OAG; yield 0.0009%) (Lavaud et al., 2000).

Cell culture, chemicals

MCF-7 cells (Michigan Cancer Foundation-**7)** were cultured in Dulbecco's Modified Eagles Medium (DMEM) (GIBCO, Invitrogen, Carlsbad, CA, US) supplemented with 10% fetal bovine serum (GIBCO), 1X PSN (GIBCO) and gentamycin (GIBCO). Cells were incubated in a humidified CO_2 incubator at 37°C. Camptothecin (CPT) and etoposide (ETO) were purchased from Sigma (St. Louis, MO, US) and dissolved in dimethylsulphoxide (DMSO).

Cell viability assay

Cells were seeded in 96 well plates. After 24 h, these were treated with respective compounds keeping the DMSO concentration less than 0.5%. After 72 h of treatment, cell viability was assessed by 3-(4, 5-dimethylthiazol-2-yl)-2,

5-diphenyltetrazolium bromide (MTT) assay. Briefly cells were washed with 1X PBS and treated with MTT for 4 h at 37°C. The precipitates were dissolved in DMSO and plates were analyzed on Thermo MULTISKAN EX plate reader at 595 nm.

DNA relaxation assay for topoisomerase I and II enzymes

Recombinant human DNA topoisomerase I and II enzymes were purchased from TopoGEN Inc (Port Orange, Florida, USA). DNA relaxation assay for human topoisomerase I was performed in the presence or absence of respective compounds by briefly incubating 100 fmol of supercoiled pBS SK(+) DNA with 50 fmol of the enzyme in a reaction buffer containing 25 mM Tris-Cl (pH 7.5), 5% glycerol, 50 mM KCl, 0.5 mM DTT, 10 mM $MgCl_2$, 25 mM EDTA and 150 µg/ml bovine serum albumin, as described previously (Ray S., et al 1998). DNA relaxation assay for human topoisomerase II was performed in the presence and absence of respective compounds by briefly incubating 100 fmol of supercoiled pBS SK(+) DNA with 50 fmol of the enzyme in a reaction buffer containing 50 mM Tris-Cl (pH 8.0), 150 mM NaCl, 10 mM $MgCl_2$, 5 mM ATP, 0.5 mM DTT and 30 µg/ml bovine serum albumin (the reactions buffers were provided with the enzyme by the manufacturer). The reactions were incubated at 37 °C for 30 minutes, loaded on 1% agarose gel and subjected to electrophoresis at 20 volts overnight. After completion of electrophoresis, gels were stained with 0.5 µg/ml ethidium bromide and viewed by Gel Doc 2000 (BioRad) under UV illumination. DNA Relaxation was assessed by monitoring the decreased electrophoretic mobility of relaxed topoisomerase of pBS SK(+) DNA. Camptothecin (CPT) and etoposide (ETO), were used as a positive control inhibitors for topoisomerase I and II, respectively.

DNA cleavage assays for topoisomerase I and II enzymes

DNA cleavage assays for topoisomerase I and II were performed in the presence or absence of respective compounds by briefly incubating 100 fmol of supercoiled pBS SK(+) DNA with 500 fmol of topo I or topo II enzyme. For topoisomerase I, the reaction buffer contained 10 mM Tris-Cl (pH 7.5), 50 mM KCl, 5 mM $MgCl_2$, 0.1 mM EDTA and 15 µg/ml bovine serum albumin. For topoisomerase II, the reaction buffer contained 50 mM Tris-Cl (pH 8.0), 150 mM NaCl, 10 mM $MgCl_2$, 5 mM ATP, 0.5 mM DTT and 30 µg/ml bovine serum albumin. The reactions were incubated at 37 °C for 30 minutes and stopped with 0.5% SDS. Enzymes were digested by proteinase K treatment. Reactions were loaded on 1% agarose gel containing 0.5 µg/ml ethidium bromide. The gel was subjected to electrophoresis at 80 volts for 3 hours. After completion of electrophoresis, gels were viewed by Gel Doc 2000 (BioRad) under UV illumination. Camptothecin (CPT) and etoposide (ETO) were used as positive control

inhibitors that stabilize topoisomerase I-DNA and topoisomerase II–DNA covalent complexes, respectively.

Immunoband depletion assay

For immunoband depletion assay MOLT-4 cells were cultured in 35 mm dishes separately in the presence of 10 μM each of CPT, ETO, DGT, and OAG, and harvested at different time points. For pretreatment immunoband depletion assay, cells were first treated with either 10 μM DGT or 10 μM OAG for 2 h and then treated with 10 μM CPT or 10 μM ETO. Equal amounts of protein were electrophoresed on SDS-poly acryl amide gel, separated proteins were transferred on to nitrocellulose membrane, and western blotting was performed using anti-topo I and anti-topo II antibodies (Santa Cruz Biotechnology, Inc. Santa Cruz, CA, USA).

Apoptosis assay

Briefly, MCF-7 cells (1×10^6/ml) were incubated with or without DGT (IC_{50}: 8.27 μM) and OAG (IC_{50}: 11.33 μM) for 24 h and 48 h at 37°C, 5% CO_2. Cells were then washed twice in PBS and resuspended in Annexin-V binding buffer (10 mM HEPES, 140 mM NaCl, 2.5 mM $CaCl_2$; pH 7.4). Annexin-V-FITC was then added according to the manufacturer's instructions (Calbiochem, USA) and incubated for 15 min under dark conditions at 25°C. PI (0.1 μg/ml) was added just prior to acquisition. Data were acquired using BD LSR Fortessa flowcytometer (Becton Dickinson, USA) at an excitation wavelength of 488 nm and an emission wavelength of 530 nm, and analyzed with BD FACS Diva software (Becton Dickinson, USA).

Detection of mitochondrial membrane potential

The mitochondria are attractive targets for cancer chemotherapy since its impairment renders cells non viable. The loss of mitochondrial potential is one of the indicators of apoptosis. The mitochondrial transmembrane electrochemical gradient ($\Delta\Psi_m$) was measured using mitochondrial potential sensor JC-1, a cell permeable, cationic and lipophilic dye. In viable cells, it freely crosses the mitochondrial membrane and forms J-aggregates which fluoresce red. In apoptotic cells, decrease in mitochondrial membrane potential prevents JC-1 from entering the mitochondria and remains as monomers in the cytosol that emits a predominantly green fluorescence (Deeb, D., et all., 2010). Therefore, the ratio of Jaggregates/monomers functions as an effective indicator of mitochondrial transmembrane potential and helps distinguish apoptotic cells from their healthy counterparts. Briefly, MCF-7 cells (1×10^6/ml) were incubated with IC_{50} concentrations of DGT and OAG for 24 h and 48 h at 37°C, 5% CO_2. The cells were then washed with PBS, and incubated with JC-1 (2 μM) according to manufacturer's protocol (Molecular

Probes, USA) under dark conditions for 15-30 min at 37°C, 5% CO_2. Cells were acquired using FACS and analyzed using FACS Diva software. CCCP was used as positive control.

Measurement of ROS generation

The effect of IC_{50} concentration of DGT and OAG on generation of ROS (12 and 24 h) was measured in cells (1×10^6/ml). After treatment, cells were washed with PBS and resuspended in PBS, and then incubated with H_2DCFDA (20 µM in PBS) for 30 min at 37°C. Subsequently, cells were again washed and resuspended in PBS. DCF fluorescence was determined by flow cytometry at an excitation wavelength of 488 nm and an emission wavelength of 530 nm by BD LSR Fortessa flowcytometer.

Western blot analysis of caspases and PARP-1 cleavage

Cells were cultured in 6 cm dishes and treated with either 8.27 µM DGT or 11.33 µM OAG for 48 h. Cells were lysed in NP-40 buffer and equal amounts of proteins were electrophoresed on SDS-polyacrylamide gel. Separated proteins were then transferred on to nitrocellulose membrane and detected using antibodies available in Apoptosis Antibody Sampler Kit (Cell Signaling Technology) by western blotting.

Cell cycle analysis using flow cytometry

Sub confluent cells were treated with IC_{50} concentration of DGT or OAG in culture medium as described above for 24 h and 48 h. The cells were then harvested, washed with cold PBS, and processed for cell cycle analysis. Briefly, 1×10^6 cells were resuspended in 300 µl of cold PBS to which 70% cold ethanol (700 µl) was added, and the cells were then incubated overnight at 4°C. After removing ethanol and washing with PBS, cells were suspended in 500 µl PBS, and incubated with 100 µg/ml RNase A for 1 h at 37°C. The cells were subsequently incubated with 50 µg/ml propidium iodide (PI) for another 30 min at 37°C in subdued light (Carpinelli et al., 2011). The stained cell suspension was analyzed with BD LSR Fortessa flowcytometer. The DNA content of 10,000 cells per sample was used to analyze the cell cycle using DNA histograms. The DNA content in the cell-cycle of the analyzed cells was calculated by MODFIT 3.0 software (Verity Software House, ME, USA).

Assessment of cell morphology

Cells (3×10^4/well) were grown in 6-well TC plates and treated with or without DGT and OAG at IC_{50} concentration for 24 h. Morphological changes were observed with an inverted phase contrast microscope (Model: OLYMPUS IX70, Olympus Optical Co. Ltd., Shibuya-ku, Tokyo, Japan) and photographs were taken with the help of a digital camera (Olympus, Inc. Japan). [See Fig-7 in manuscript]

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