1 **Supplementary Figures**

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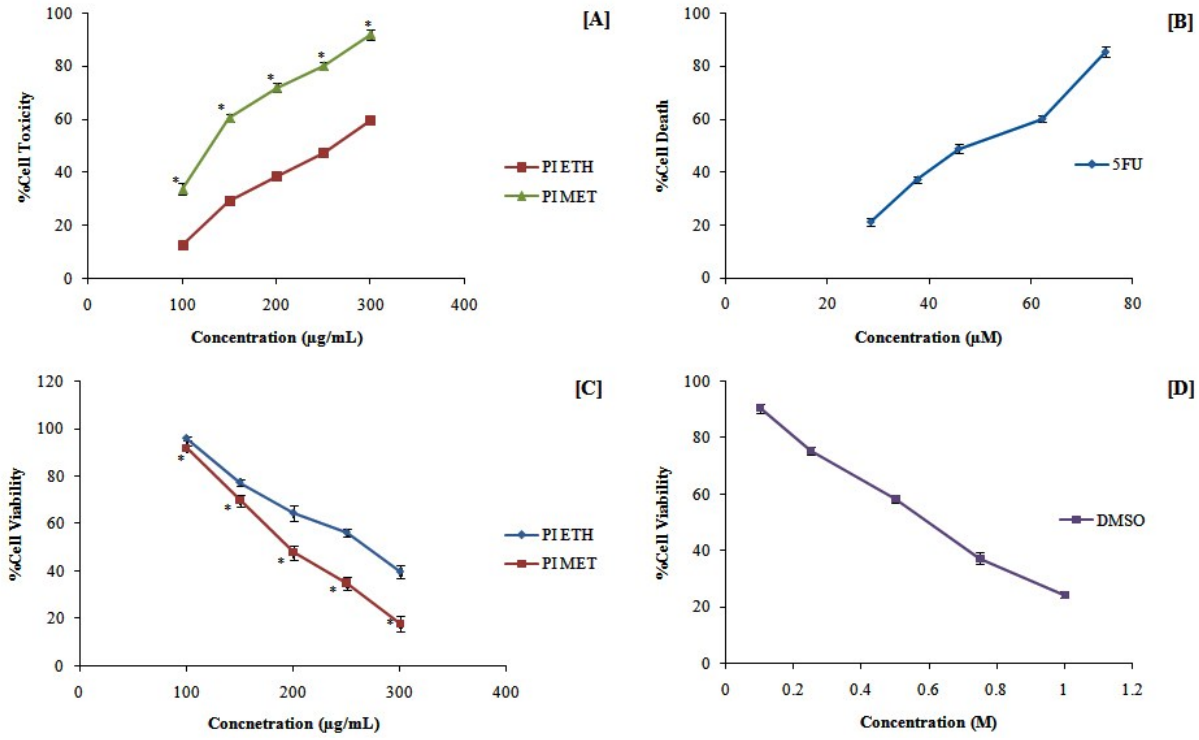

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4 **Supplementary Figure S1.** Cytotoxic effect determined by MTT and LDH release assays
5 against HT29 colon cancer cells. [A & B] Cytotoxic effect of PI extracts and 5-fluorouracil
6 determined by MTT assay. [C & D] Cytotoxic effect of PI extracts and DMSO determined by
7 LDH assay. Each value represents mean ± SD from triplicate measurements. $p \leq 0.05$ considered
8 as significantly different.*Methanol extract (PIMET) significantly different from ethyl acetate
9 extract (PIETH).

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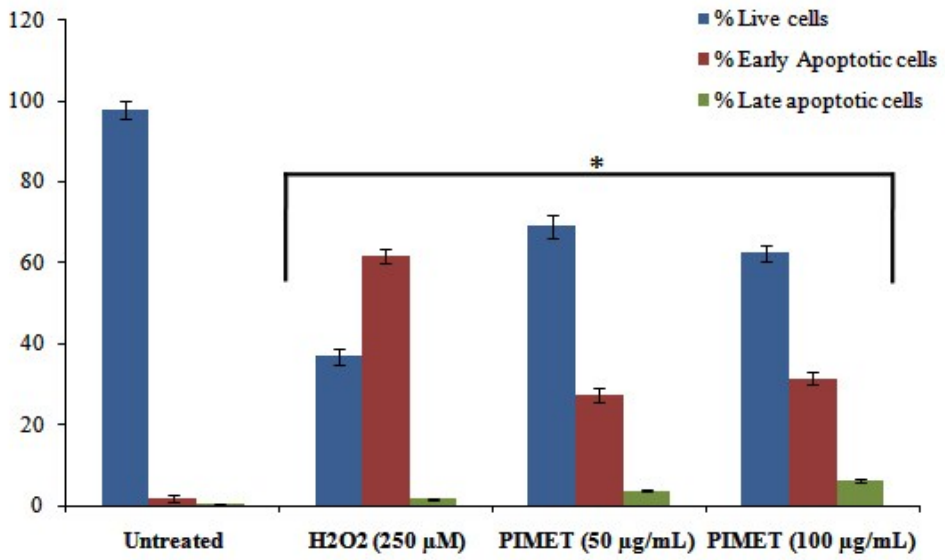
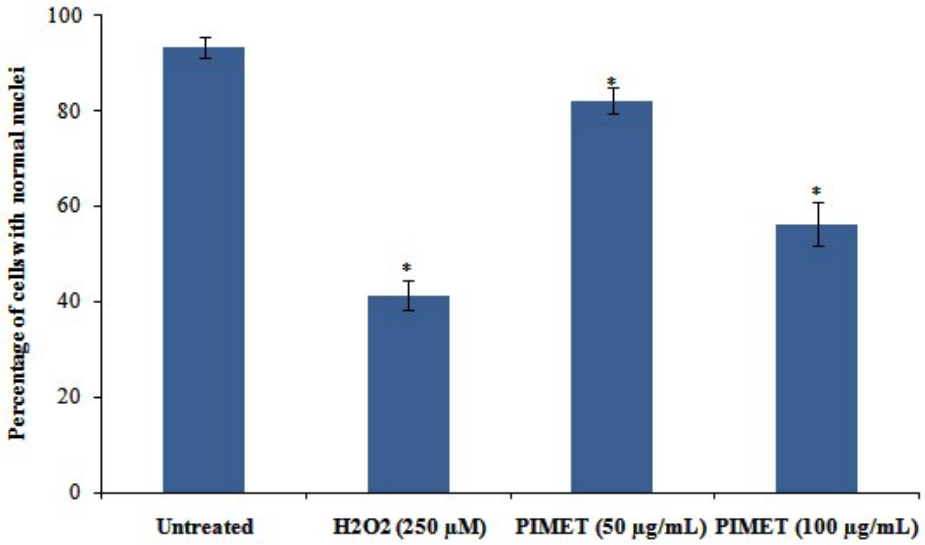


Supplementary Figure S2. Quantification of the live, early apoptotic, and late apoptotic cells. Tests were done in triplicate, counting a minimum of 200 total cells each in three different fields. *significantly different from untreated control.



Supplementary Figure S3. Quantification of the cells with normal nuclei. Tests were done in triplicate, counting a minimum of 200 total cells each in three different fields. *significantly different from untreated control.

Method

Detection of cell death by acridine orange/ethidium bromide staining

The effect of PI extract to induce cell death in HT29 cells was determined by AO/EtBr dual staining (Pitchai et al. 2014). The cells were grown on the 96 well black plate, and then treated with the different concentration of PIMET for 24 h. After incubation, 5 µL of AO (1 mg/mL) and 5 µL of EtBr (1 mg/mL) were added, and the induction of cell death was observed by using confocal microscopy (BD Pathway 855, BD Bioscience, USA). The percentages of viable, early apoptotic, late apoptotic and secondary necrotic cells were determined by counting 200 cells in three different fields in triplicate.

Hoechst 33342 staining

Apoptotic activity of extract on HT29 cells were determined by Hoechst 33342 staining as described by Harada et al. (2005) with slight modification. 1×10^4 cells were treated with different volumes of PI extract and incubated for 24 h. Control group cells treated with media containing 0.1% DMSO and H_2O_2 (250 µM) was used as positive control. Cells were washed with PBS and fixed with 70% ethanol for 5 min. After fixation, cells were incubated with Hoechst 33258 stain in PBS (5 µg/mL) for 30 min at 37°C in the dark. Cells were thoroughly washed with PBS and examined under confocal microscope (BD Pathway 855, BD Bioscience, USA) with an excitation of 350 nm and emission of 460 nm. Apoptotic cells were identified by nuclear condensation and apoptotic bodies. The percentage of cells with normal nuclei was determined by counting 200 cells in three different fields in triplicate.

Detection of mitochondrial membrane potential in HT29 cells

The effect of PI extracts on mitochondrial membrane potential was identified by staining with Rh123, a green fluorescent cationic dye that binds to polarized mitochondrial membrane and accumulates as aggregates in the mitochondria of normal cells. When there is reduction in mitochondrial membrane potential, the dye washes out resulting in reduction of green fluorescence. For analysis of changes in mitochondrial trans-membrane potential, HT29 cells were treated with the different concentrations of PI extract for 24 h. Following the treatment, the cells were directly incubated with Rh123 (1.6 mM) for 20 min in the dark, followed by rinsing with several changes of PBS. Data analysis was performed using FACS. A reduction in Rh123 green fluorescence indicates reduced mitochondrial membrane potential.

Adenisone triphosphate (ATP) production by HPLC analysis

The ATP levels in HT29 cells after treatment with extract was determined using HPLC method (Hahn-Windgassen et al., 2005). After treatment, the cells were trypsinized and centrifuged at 800×*g* for 3 min and the pellets were suspended in 4% perchloric acid on ice for 30 min. The pH of the lysates was adjusted between 6 and 8 with 2 M KOH. Precipitated salt was separated from the liquid phase by centrifugation at 13000×g for 10 min at 4°C. ATP was quantified on a Prominence HPLC system (Shimadzu, Japan) containing LC-20 AD system controller, Phenomenex Gemini C18 column (250×4.6 mm, 5 µm), a column oven (CTO-20A), a Rheodyne injector (USA) with a loop of 20 µL volume and a diode array detector (SPD-M20A). A buffer 20 mM KH_2PO_4 and 3.5 mM K_2HPO_4 $3H_2O$ (pH 6.1) was used as the mobile phase. The flow rate was 1 mL/min, the injection volume was 20 µL and column was at 37°C. The fractions were monitored at 259 nm. Sample peaks were identified by comparing with retention times of standard peaks and by spiking with standards. LC Lab Solutions software was used for data acquisition and analysis.

Detection of Cytochrome C release

Cytochrome C release due to mitochondrial damage was assessed according to Radhakrishnan et al. (2007) with slight modifications. After the treatment of HT29 cells with PI extract, cells were trypsinized and suspended in 50% acetonitrile solution containing 0.1% trifluro acetic acid (TFA) and kept at room temperature for 10 min with mild intermediate mixing. Then the mixture was centrifuged at 5000 rpm for 10 min to precipitate high molecular weight plasma proteins. Cytochrome C content in the supernatant was quantified on a Prominence HPLC system (Shimadzu, Japan) containing LC-20 AD system controller, Phenomenex Gemini C18 column (250×4.6 mm, 5 µm), a column oven (CTO-20A), a Rheodyne injector (USA) with a loop of 20 µL volume and a diode array detector (SPD-M20A). A gradient system consisting of solvent A- 0.1% TFA in water: 0.1% TFA in acetonitrile (70:30) and solvent B - 0.1% TFA in water: 0.1% TFA in acetonitrile (64:36) was used as mobile phase achieving gradient range 0-100% B in 30 min. The flow rate was 1 mL/min, the injection volume was 20 µL and column was at 37°C. The fractions were monitored at 393 nm. Sample peaks were identified by comparing with retention time of standard peak. LC Lab Solutions software was used for data acquisition and analysis.

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