

Figure W1. L + E + C + P inhibits growth, stimulate adhesion, inhibits migration, and inhibits chemotaxis toward SDF1α of hormone-independent DU145 cells. (A) DU145 cells were treated with four PJ components L + E + C + P at 4 and 8 μ g/ml and counted for increasing times after initiation of treatment. Controls represent no treatment. The medium containing PJ components was changed daily. Bars represent SEM. ***P < .001; **P < .01. (B) PC3 cells were treated with L + E + C + P at 4 or 8 μ g/ml and the percentage of dead cells was determined by Trypan blue staining at 12, 24, 48, and 72 hours. (C) DU145 cells were plated on gelatin-coated dishes, and 24 hours later, medium was changed and the cells were treated with PJ components L + E + C + P at 4 and 8 μ g/ml. We tested for adhesion to the substrate at 12 and 24 hours after initiation of treatment by recording the time it took for trypsinization to remove all of the cells from the dish. Control represents no treatment. Within each experiment, the times of trypsinization were the same within 1 minute for each specific treatment. (D) DU145 cells were treated with PJ components L + E + C + P at 4 and 8 μ g/ml for 72 hours, and the distance migrated by the cells from the wounded edge to the leading edge was measured at the indicated time points. Controls represent no treatment. The medium containing PJ components were changed daily. (E) DU145 cells were allowed to attach to the top of the filter of the chemotaxis chamber for 4 hours and then treated with PJ components L + E + C + P at 4 and 8 μ g/ml for 12 hours. At this time, 100 ng/ml SDF1α were introduced into the lower chamber and the cells found on the bottom of the filter were counted 3.5 hours later. Control had no treatment. The number of cells found on the underside of the filter was counted 3.5 hours later. Bars represent SEM. ***P < .001; **P < .01; *P < .05.

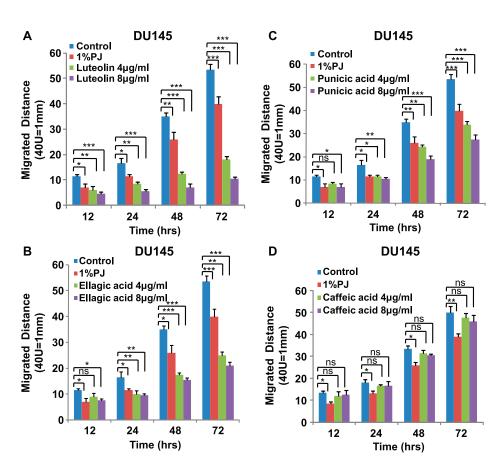


Figure W2. Luteolin, ellagic acid, and punicic acid but not caffeic acid individually inhibit cell migration of hormone-independent DU145 cells. DU145 cells were treated with individual PJ components (A) luteolin, (B) ellagic acid, (C) punicic acid, and (D) caffeic acid at 4 and 8 μ g/ml for 72 hours, and migration assay was performed as described in Figure 1. SEM. ***P < .001; *P < .01; *P < .05.

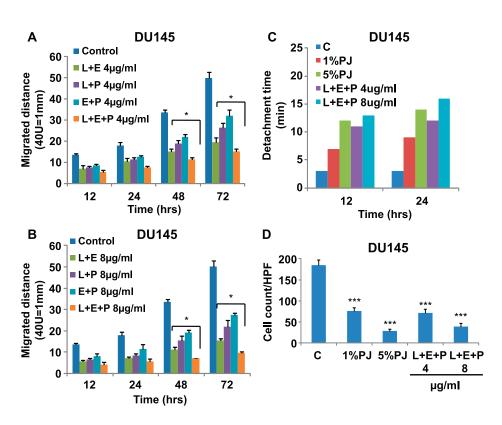


Figure W3. L + E + P is the most potent combination to inhibit cell migration and its effect on cell adhesion and chemotaxis toward SDF1α of hormone-independent DU145 cells. DU145 cells were treated with different combination of PJ components L, E, and P at (A) 4 and (B) 8 μ g/ml for 72 hours, and migration assay was performed as described in Figure 1. SEM. ***P < .001; **P < .01; *P < .05. (C) DU145 cells were plated on gelatin-coated dishes, and 24 hours later, the medium was changed and the cells were treated with PJ components L + E + P at 4 and 8 μ g/ml. Adhesion assay was performed as described in Figure 1. (D) DU145 cells were allowed to attach to the top of the filter of the chemotaxis chamber for 4 hours and then treated with PJ components L + E + P at 4 and 8 μ g/ml for 12 hours. Chemotaxis assay was performed as described in Figure 1. Bars represent SEM. ***P < .001; **P < .01; *P < .05.