# **Supporting Information**

# Characterization of *in Vitro* ADME Properties of Diosgenin and Dioscin from *Dioscorea villosa*

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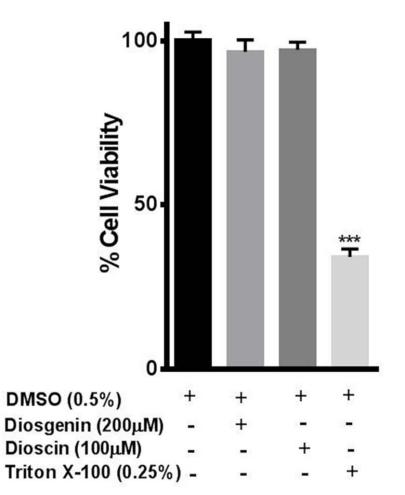
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# **Methods and Results:**

#### Determination of diosgenin and dioscin cytotoxicity in Caco-2 cells:

To determine if diosgenin and dioscin were cytotoxic to Caco-2 cells, CellTiter 96<sup>®</sup> AQ<sub>ueous</sub> one solution cell proliferation assay (Promega Corporation) was used according to the manufacturers guidelines. Briefly, 20,000 cells per well in 100  $\mu$ L DMEM media were seeded in 96-well plates. After 24h, diosgenin (200  $\mu$ M), dioscin (100  $\mu$ M), and positive control Triton X-100 (0.25%) were added. After 2h incubation at 37 °C, 20  $\mu$ L of MTS [3-(4,5- methylthiazol-2-yl)-5-(3- carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium salt] dye was added and incubated for 1h at 37 °C. After 1h, the absorbance of the plate was read at 490 nm on a Spectramax M5 plate reader. Percent cell viability was calculated compared to the control. No cytotoxicity was observed with either diosgenin (200  $\mu$ M) or dioscin (100  $\mu$ M) in Caco-2 cells, while Triton X-100 (0.25%) showed significant cytotoxicity (**Figure 1S**). These results suggest that the concentrations of diosgenin and dioscin used in bi-directional transport experiment had no effect on the viability of Caco-2 cells.

**Figure 1S**: Cell viability of Caco-2 cells in response to diosgenin and dioscin (2 h treatment) as determined by MTS assay. Triton X-100 (0.25%) was used as positive control. \*\*\* p<0.05, determined by Kruskal-Wallis test. The data shown are mean  $\pm$  SD of triplicate treatments in one experiment.



# Figure 1S