Reviewer's report

Title: Gallotannin-rich Caesalpinia spinosa fraction decreases the primary tumor and factors associated with poor prognosis in a murine breast cancer model

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Reviewer: Kent Hunter

Reviewer's report:

This manuscript describes the in vitro and in vivo characterization of the P2Et extract, derived from Caesalpinia spinosa, on breast cancer progression. Using the 4T1 mouse mammary tumor model the investigators demonstrate that P2Et induces apoptosis in cultured cells, presumably through the mitochondrial apoptosis pathway. Furthermore they demonstrate that P2Et has effects on IL-6 secretion in both in vivo and in vitro systems. Finally they demonstrate that treatment of orthotopic tumors with the P2Et extract results in a decrease in primary tumor growth and dissemination of tumor cells into the spleen.

Concerns:

Major Compulsory Revisions

Figures 3&5: The investigators have demonstrated in figure 3 that treatment of 4T1 cells in vitro results in an up regulation of the pro-progression cytokine IL-6, but in vivo there is a decrease in IL-6 in the animal serum. How do the investigators account for this discrepancy? Some discussion of this opposing phenotype seems to be appropriate.

Figure 1 b: There are two sets of FACS plots for this panel. What do the two sets represent? 24 and 48 hour treatments? The figure legend states only 48 hours.

Figure 3 legend: IL-6 should be labeled as panels a & b, not a & c, MCP-1 should be c & d, as indicated on the figure

Figure 5: Please be certain to provide high resolution photomicrographs for the manuscript.

The data presented in figure 5 also suggests that the in vivo anti-IL-6 activity of P2Et may not be responsible for the anti-metastatic effect, at least in the lung. 9.3 mg/kg of P2Et is sufficient induce all of the measured in vivo phenotypes, but does not appear to have an effect on pulmonary metastases. In fact, if these photomicrographs are representative, the opposite might be the case. Due to the quality of the images it is not possible to assess this possibility in the other organ tissues. Splenic metastasis is also relatively rare in breast cancer, so it is not clear how significant an effect this treatment might be in the clinical setting, particularly since it appears to be single cell dissemination rather than macroscopic metastasis that is effected.
Minor essential revisions

Page 9: clonogenic assays are routine assays used for tumorigenesis. However, no in vitro assay has been found to be particularly predictive of metastatic capacity. Therefore extending the results of the clonogenic assay to predict the terminal stages of cancer is probably questionable.

Page 11, last sentence of the first paragraph. Please clarify.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

'I declare that I have no competing interests'