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

Version: 2 Date: 17 October 2012

Reviewer: Suranganie Dharmawardhane

Reviewer's report:

Major compulsory revisions:

1. On page 7, the authors state that toxicity studies were conducted with 1-8mg/ml P2Et fraction. In the tumor model, the mice were injected with 9.3 or 18.7 mg/kg, how does this compare with the toxicity data? Please, show the LD50 data in a supplemental figure using the same units.

2. The IC50 for cytotoxicity is given in µg/ml in Table 1. Please, show a dose response curve for how the IC50 was calculated for P2Et in 4T1 cells. Moreover, given that doxorubicin gave an IC50 of 0.5 µg/ml and the P2Et fraction gave an IC50 of 34.1 µg/ml, it is misleading to start the Results section with the heading “P2Et fraction is highly cytotoxic to 4T1 tumor cells” when the effects on cytotoxicity are modest.

Please, clarify the disparity in data from your previous study (reference 12) where you show IC50 of 64 µg/ml for fibroblasts and an IC50 >125 µg/ml for fibroblasts in Table 1.

3. Figure 5: The quality of the micrographs is poor and it is difficult to see what the authors call “metastatic infiltration”. The white arrows mask the cells stained with H&E. In this model, in 30 days the lungs should show clear metastatic foci not just infiltration (see Int J Exp Pathol. 2007, 88:351-560). Given that these are more than micrometastases in the lung, please, clarify how you counted individual cells. Also in the example in response to 9.3 mg/kg P2Et, there may be more metastatic cells in the lung compared to control.

Minor essential revisions:

1. Abstract: Methods
   In the sentence “The present study evaluated cytotoxic, antitumor, and tumor progression activities of a gallotannin-rich fraction derived from Caesalpinia spinosa (P2Et).”, “tumor progression” should be changed to “anti-tumor progression”.

2. Abstract: Results, Define IL-6.

3. Introduction First paragraph, last sentence, “induces apoptosis in the murine and human
breast cancer cells MCF-7 and S115, respectively [9].” Should be “induces apoptosis in the murine and human breast cancer cells S115 and MCF-7 respectively [9].”

4. Page 5, Heading, Please define MMP as mitochondrial membrane potential.

5. Ethanol is used as the negative control. Please, state the % of ethanol used throughout the manuscript or it will be assumed that you used 100% Ethanol.

6. Please, give the number of animals/group in Figures 4 and 5. The methods say that the N=7-8 but the experiments were conducted twice and the results are the mean. Therefore, is the N, 14 or 16?

7. Fig. 2A shows nuclear segmentation not DNA fragmentation which is usually shown by agarose gel electrophoresis.

8. The clonogenic assay as shown by cell clonogenicity is a measure of cell survival and proliferation. As the authors state, to use it as a “golden standard for antitumor activity and metastasis” the assay should be conducted with tumor cells recovered from metastatic foci, that can be grown in a clonogenic assay.

9. Please, edit typos and grammar mistakes throughout the document. For instance, “mice mammary fat pads should be “mouse mammary fatpads”. In Fig1 a, MM-Potencial should be “MMP” or mitochondrial membrane potential”. Fig. 4 caption, “tumor weight” should be “weight”, etc. Also in the Figures, decimal points designated by a “comma” (i.e. 3,4) should be designated by a “period” (3.4).

10. Please, give method for leukocyte counts in Methods section.

11. Figure 3, Please, change caption to indicate that a,b represents results for IL-6 and c,d for MCP-1.

Discretionary revisions

1. Migration/invasion assays in response to P2Et are been better indicators of metastatic efficiency of cells in tissue culture than the clonogenic assay.

**Level of interest:** An article of importance in its field

**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 have no competing interests.