Reviewer's report

**Title:** Anti-proliferative and anti-adhesive effects of four plant extracts on the breast cancer cell line MCF-7

**Version:** 2  **Date:** 12 April 2014

**Reviewer:** Hsueh-Wei Chang

**Reviewer's report:**

Major comments:

1. [page 10]

The authors mentioned that “(Table 3). Treatment with 10 µg/ml JCP1 and JCP2 resulted in the highest levels of dead MCF-7 cells (56% and 87%) which are positive for the PI labeling. These values are comparable with the apoptosis data obtained from the determination of the sub-G1 phase in the cell cycle measurement which indeed showed the highest percentages in sub-G1 phase.”

However, I still have several concerns as follows:

1. However, the description of “apoptosis data obtained from the determination of the sub-G1 phase” is not suitable. The sub-G1 population cannot complete reflect the apoptosis. I will suggest the author to unlike the sub-G1 and apoptosis.

2. I agree with the authors’ findings that the PI positive is consistence with sub-G1 population. However, the both PI/Annexin V double positive and annexin V positive alone are weak compared to control. These results indicate that the flow cytometric changes are mainly on PI positive alone. The rationale for annexin V/PI flow cytometry is provided in “Fig. 2D, Fig. 4D and page 11 in Lipids in Health and Disease 2013, 12:36 (http://www.lipidworld.com/content/12/1/36)” as follows:

   1. Annexin V positive and PI negative cells are regarded as early apoptosis.
   2. Annexin V/PI double positive cells are regarded as late apoptosis.
   3. Annexin V negative and PI positive cells are regarded as necrosis.

(3) Therefore, I will suggest the authors to describe their result as follows: The sub-G1 population is increase in consistence with the PI intensity of PI/Annexin V flow cytometry, indicating the necrosis may be the possible reason for cell death.

4. [Discussion]

The author should discuss the finding that drug treatment leads to sub-G1 but no apoptosis based on the reference as described above. Some literature for necrosis research may be discussed in the section Discussion.

Minor comments:

1. [page 10]
“But ultimately, all four extracts mediate a significant increase in sub-G1 phase. The extracts JCP1 and JCP2, already at the low concentration of 10 µg/ml caused a significant increase in the apoptotic phase”

# Change the “apoptotic” to “sub-G1” as follows:

“But ultimately, all four extracts mediate a significant increase in sub-G1 phase. The extracts JCP1 and JCP2, already at the low concentration of 10 µg/ml caused a significant increase in the “sub-G1” phase”

2. [Table 3] The value should be provided in mean+_ SD (n = ?). Now, it look like one experiments.

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

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests' below