Reviewers report

Title: Cytotoxicity Screening of Bangladeshi Medicinal Plant Extracts on Pancreatic Cancer Cells

Version: 1 Date: 26 April 2010

Reviewer: Po S Leung

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This paper is to screen cytotoxicity from 56 extracts of 43 unique medicinal plants from Bangladesh against pancreatic cancer using its cell lines. The author used a label-free PC biosensor-based assay, and they found that nine extracts exhibited significant cytotoxicity. MTT assay on two additional pancreatic cell lines (Mia-Paca2, and Capan-1) further confirmed their cytotoxicity. Finally, the potential mechanism by which the extract of Petunia punctata induces apoptosis was examined using caspase-3 Colorimetric Assay.

There has been a paper published in Sensors and Actuators B (vol. 132, 2008, 418-425) which screened cytotoxicity of 61 extracts of medicinal plants from Bangladesh against MCF-7. In this paper, the plant extracts include 56 extracts, and label-free PC biosensor-based assay was also employed. In general, this paper lacks of novelty and originality. In addition, only extracts but no pure compounds were used while no convincing mechanism(s) was proposed. In term of methodology, the three-tiered screening system (PC assay-MTT assay-caspase-3 colorimetric assay) appears to be too simple. Nevertheless, pancreatic cancer is a malignant disease with highly mortality, and it is highly resistant to cytotoxic agents. Thus the purpose of this research would add, to some extent, advance in this field.

Major comments:

1. The numbering of figures (pink) is confusing with some mistakes, making it to be difficult to understand.

2. P. punctata treated cells can induce cellular apoptosis. It needs additional experimental results to further confirm apart from using caspase-3 colorimetric assay. For example: morphological changes observed by fluorescence staining, flow cytometry (Annexin V kit), ELISA apoptosis assay should be included in this paper.

3. West blot analysis should also be used so as to confirm some proteins of interest, such as apoptosis-inducing ones. In this version, only caspase-3 colorimetric assay was examined.

4. In the discussion, two relevant papers (Lau ST et., Br J Cancer 102, 583-593, 2010 and Lau ST et., Cancer Letters 281, 42-52, 2009) need to be cited so as to further support the potential use of herbal medicine for the treatment of pancreatic cancer.
Minor comments:
1. Mark the top nine extracts showing the significant cytotoxicity in table1.
2. “Figure 1d shows PWV shift images of an extract-treated well where nearly100% cell death was observed”. Please make clear which extract treated cell was done? (P. punctata, No. 34 ) (page 9, line 15).
   “Figure 1e shows PWV shift images of an extract-treated well where the extract enhances cell proliferation.” Again please make clear which extract treated cell was done? (A. glabra, No. 11)
3. Four of these extracts induced over 50% cell death in the MIA and Capan-1 cell lines (Figure 3). (Mark extracts number No.4, 6, 34, 41) (page 10, line 6)
4. Page 10, line 10, “extract” is not in italic type. Line 12, “A. sessilis” is in italic type.
5. Page 10, line 21, “an MTT assay” should be “an caspase-3 Colorimetric Assay”
6. Page 11, line 1, “the compound” should be “the extract”.
7. Page 11, line 4, “Staurosporine and Curcumin exhibited the same level of cytotoxicity on Panc-1 cells as measured through an MTT assay.” Give the exact data of Staurosporine treated Panc-1.
8. Page 11, line 7, “Figure 4b” should be “Figure 5b”
9. Page 8, line 9, “the plant extract” change to “Petunia punctata extract (100µg/ml)”, and give the Staurosporine concentration.
10. Page 12, line 19, “punctata” should be in italic type.
11. Solanaceae family is not in italic type. The same is true as “Amaranthaceae family”.
12. Page 22, line 6, “P. punctata” and “A. glabra” should be in italic type.
13. The name of plant species in table 1 should be in italic type.