Author's response to reviews

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

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Version: 3 Date: 22 July 2010

Author's response to reviews: see over
SUB: Manuscript revisions

Please consider our revised manuscript (no. 1348472814366240) titled “Cytotoxicity Screening of Bangladeshi Medicinal Plant Extracts on Pancreatic Cancer Cells”.

This work aims to investigate a library of 56 extracts from 44 unique medicinal plants of Bangladesh for their cytotoxic effects on three pancreatic adenocarcinoma cell lines using a label-free biosensor assay and the colorimetry-based MTT assay. We found that crude extracts of *Petunia punctata*, *Alternanthera sessilis*, and *Amoora chittagonga* showed cytotoxicity to the cancer cell lines with IC$_{50}$ values ranging between 13.08 and 49.8 µg/mL. Furthermore, treatment of Panc-1 cells with the *Petunia punctata* extract was shown to increase caspase-3 activity, indicating that the observed cytotoxicity was mediated via apoptosis.

We have made recommended changes in the manuscript (highlighted in yellow) relative to most of the questions posed by the two reviewers and these changes discussed individually below. We thank the reviewers for their helpful input and hope that these revisions sufficiently address all comments.

(Reviewer comments in italics; Responses in red)

**Reviewer 1 (Po S Leung):**

**Major comments:**

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

**Response:** We have combined the two parts of Figure 1 and thus eliminated the mistake in the numbering.

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.*
Response: Unfortunately, we are unable to address the above two comments by performing additional experiments. We received only a small quantity of *P. punctata* from our contact (Dr. R. Chowdhury) in Bangladesh and have exhausted our sample. In addition we have been unsuccessful in obtaining additional quantities of this extract as Dr. Chowdhury has relocated to the UK. We are thus in a difficult position such that we are unable to perform the additional experiments requested by Dr. Leung. We would provide such data were we to have sufficient quantities of the extract.

However, we feel that in spite of the absence of these additional experimental results, that this manuscript in its revised form per the reviewer comments provides useful information to the scientific community of complementary medicine. The focus of this work has been on rapid exploration of the cytotoxic effects of a collection of medicinal plant extracts on pancreatic cancer using the label-free photonic crystal assay. While we have identified *P. punctata* as one of three extracts with high cytotoxicity to three pancreatic cancer cell lines, we have also shown that this extract shows similar levels of toxicity to a normal foreskin cell line. These results appear to indicate that *P. punctata* shows low selectivity for cancer cells, thus reducing interest in the further investigation of this extract as a potential therapeutic candidate. We hope you will agree that additional experiments to confirm apoptosis induction would not provide any new crucial information.

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.

Response: We have included an opening sentence in the discussion section that references the above two papers (highlighted in yellow). Additionally, the paper “Lau ST et., *Cancer Letters* 281, 42-52, 2009” has been referenced in the introduction both in the original version of this manuscript as well as the current revised version.

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, “Staurosprine and Curcumin exhibited the same level of cytotoxicity on Panc-1 cells as measured through an MTT assay.” Give the exact data of staurosprine 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 Staurosoprine 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.

Response: All minor changes have been made (highlighted in yellow).

Reviewer 2 (Ramzi Mothana):

1- The section "Plant extracts" should be divided into 2 parts, namely plant materials including the first part and "Extraction of plant materials” including the extraction details.

Response: We have now divided the plant extract section into two sections titled “Plant materials” and “Extraction of plant materials”.

2- I suggest adding another table representing the full names of the plants (complete species names with authority and families), voucher numbers, parts used (roots, leaves or fruits, etc….) and can be considered as table 1.

Response: We have updated Table 1 to include complete species name with family name. For all plants tested here, the leaves were used to prepare the extracts and this information has now been included in the section titled “Extraction of plant materials”. We do not presently have a complete list of voucher numbers.

3- Table 1
A- The title of this table is to long. So rewrite it again and let the other details as appendix or footnote at the end of the table.

Response: We have shortened the title and included a footnote as suggested.

B- Names of the plant species must be written in italic.
Response: We have made this change in the manuscript.

C- What is the difference between extract 12 and 13?? Both were of the same
plant and extracted with alcohol methanol and ethanol. I believe that there is no a big difference in the polarity to have completely different extracts. So please explain why did you use both solvents and also how comes that the activity so different??

Response: We found that Table 1 had an error in the plant species name for extract 13. The extract 13 name should read Brunfelsia americana instead of Anogeissus latifolia. Thus extracts 12 and 13 were not the same extract. This has been corrected in the revised manuscript and accordingly the total number of unique plants screened has been updated to read 44 instead of 43. Additionally, the identity of the extracts 12 and 13 can also be verified in our previously published paper (Sensors and Actuators B, vol. 132, 2008, 418-425) where the same set of 56 extracts (and an additional five extracts) were studied. Table 1 of that paper lists the extract numbers (the internal identification numbers used in our lab) and plant species names.

2- Extract 54 and 55.
Same plant was extracted with the same solvent and different cytotoxic activities were found?? Explain??

Response: We have been unable to explain the difference in activity observed for extracts 54 and 55 (same plant extracted with the same solvent). However, we would like to note that similar differences in cytotoxicity has been observed on between the same two extracts when tested on a breast cancer cell line (MCF-7) in our previously published paper (Sensors and Actuators B, vol. 132, 2008, 418-425). One possible explanation might be that two plant samples from two different regions may have been used to prepare these two extracts. However, we have not been able to verify this with the person that provided our lab with the plant extracts.

C- Extract 21 and 39
The type of the extract (chloroform or methanol or etc….in column 3) must be rewritten in the same line of the plant.

Response: We have made the recommended formatting change.

We thank both reviewers for their insightful comments and hope that all questions raised have been clearly addressed.

Thanking you,
Sincerely
Kenneth L. Watkin
(Corresponding Author)