Reviewer's report

Title: Identification of Cassia tora Trypsin inhibitor active against proteases of Aspergillus flavus and Bacillus sp.

Version: 2 Date: 15 April 2011

Reviewer: Jelena Radosavljevic

Reviewer's report:

Major Compulsory Revisions

1. Authors have to reconsider changing a title “Identification of Cassia tora Trypsin inhibitor active against proteases of Aspergillus flavus and Bacillus sp.” into something that more fits the objectives of their research (maybe: Report on protease inhibitory substances in Cassia tora seeds)

2. There is no clear evidence on isolation or at least partial purification of trypsin inhibitor. All tests (dot-blot, spectrophotometric, antifungal) were run by using full plant extract, not a fraction after ammonium-sulphate precipitation or SEC on Sephadex.

3. Conditions for testing on presence of protease inhibitors are inconsistent. For all tests was used heat-treated extract (with no reference for choosing temperature of 55 C and 15 min) and for activity gel staining it is not clear what is used (I suppose it is fraction after SEC on Sephadex, with no heat treatment).

4. There is no reason for showing SDS-PAGE and fractions of partially purified potential inhibitor when that part of the work does not correlate with results obtained from different protease assays. Also, there is no clear evidence that proteins that correspond to the bands around 18 kDa (not one band as authors suggest) is the same one that is involved in inhibition of all proteases mentioned in the work. Also, that sample has not been heat-treated. In order to claim that 18kDa protein(s) is(are) trypsin inhibitors, all tests should be repeated by purified protein.

5. Results from dot-blot with bacterial and fungal protease are not shown in Fig1, and in order to claim that there is inhibitory activity in extract data have to be shown with appropriate controls.

6. Results on inhibition of sporulation should be represented as table or figure.

7. Some statistical test should be performed in order to claim if there is statistically significant difference between activity of trypsin with and without extract (and for all other sets of proteases) and also it would be very nice to compare if there is statistically significant difference among inhibition of all proteases used in study (trypsin and bacterial and fungal proteases).

I strongly suggest rewriting the manuscript and choosing in which way results have to be interpreted: as report on inhibitory activity in extract of Casia tora seeds’ extract or identification of trypsin inhibitor present in extract. The second
option requires purification of inhibitor and thorough investigation of inhibition of proteases.

Minor Essential Revisions
1. Changing Tris-Cl in Tris-HCl
2. Insert reference for defining inhibitory unit.
3. For dot-blot assay find more suitable term. Dot-blot is considered as blot performed on membrane probed with antibodies. There are no antibodies used in this study, hence, more appropriate term might be spot-test or something similar.
4. For activity gel staining (if it is chosen to go with identification of inhibitor) report concentration of Tris-HCl for buffer and activity of trypsin.
5. Dimension of chromatographic columns are expressed in mm (internal diameter x height)
6. It is not clear which test was used to select fractions from protein purification that have the highest concentration of inhibitors (by spot test, spectrophotometric or something else). If authors want to insist on identification of inhibitor, SDS-PAGE that correspond to each purification step and inhibition of each fraction have to be shown.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

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.