Reviewer's report

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

Version: 2 Date: 24 April 2011

Reviewer: Alvin Ibarra

Reviewer's report:

This report describes the evaluation of the protease-activity inhibitory capacity of fractions from Cassia tora (Senna tora).

The study is of interest in this scientific field, but the manuscript needs improvement before its publication.

Major Compulsory Revisions

Abstract

1st Paragraph (Ph) - Define "multiple therapeutic applications"
1st Ph - It is recommended using the complete scientific name in the entire document: Cassia tora.
1st Ph - It is stated that Cassia tora seeds have been effectively reported in treatment of skin and gastrointestinal disorders. It is confusing if this is a statement on the traditional use of the plant or if there is any relation with the protease activity.
1st Ph - Aims needs to be defined according to the methods used.
2nd Ph - Why the seeds of Cassia tora were washed...? A small explanation of the process' rationale is advised.
2nd Ph - "Spectrophotometric methods" is too ambiguous, a better definition is recommended.
2nd Ph - Define SDS-PAGE [dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) ?] 2nd Ph - Which inhibition fraction was used?
2nd Ph - Which statistical method were used; otherwise, clearly explain which criteria you used to define differences.
3rd Ph - Were differences statistically significant?
3rd Ph - You can use the acronym SDS-PAGE here instead of the full name. What PI stands for? Protease Inhibitor?
4th Ph - The word "excellent" is subjective.

Background

1st Ph - This paragraph is too vague, needs clarification and be supported by references. The evidence on traditional use needs to be stressed with well
supported citations.
2nd Ph - First sentence needs reference.
3rd Ph - Sentences need references.
3rd Ph - Define the aflatoxine of aspergillus (B1).
3rd Ph - It is recommended separate the paragraph in two, one related to aflatoxin B1 in plants, and another regarding its effects on human health.
4th Ph - Sentences need references.
5th Ph - Lack of references.
5th Ph - The aim is not well explained, in the way it is written is more a conclusion that an objective. Moreover, it needs a better explanation. First of all, the report has identified a fraction from Cassia tora with protease inhibitory activity, and not characterized the compound responsible of this action; therefore, the term "a protease inhibitor (PI) has been identified" is misleading.
5th Ph - It is recommended to explain the use of Traditional Indian Medicine and the definition of Cassia tora at the beginning of this section, and leave the aim of the research work at the end of the section.

Methods

Plant material and proteases...
1st Ph - It is recommended to create a section only for plant material and extract preparation. It is important to explain the location where the plant was harvested, how it was treated after collection, and if a voucher specimen was deposited in a herbarium, in this case a voucher number has to be mentioned.
1st Ph - Similar criteria for obtaining bacterial, fungal and animal proteases.
1st Ph - Check the units (i.e. along the document is used ml and mL indistinctly).

Detection of protease inhibitory activity

(A) Dot-blot method
1st Ph - First sentence is confusing: "Trypsin.... containing ... trypsin)"

(B) Spectrophotometric method
1st Ph - The name of this method is too generic and needs better definition.
1st Ph - Please re-write the sentence "residual protease activity of each protease...." and add a reference to this sentence.

Antifungal activity assay
1st Ph - Please, add the conditions used to inactivate PI (control).

Statistical analysis
1st Ph - SD was applied to all experimental results (i.e dot-blot tests)? This needs clarification. Some experimental results must be treated with better statistical analysis; for instance, results in Figure 2 can be analyzed by ANOVA.
Results and Discussion
1st Ph - The introduction is irrelevant in this section, this paragraph should go in the Background.

Dot-blot analysis
1st Ph - The comparison is subjective, the inclusion of a quantitative measurement and statistical analysis in the dot-blot test is advised.
2nd Ph - The sentence about the thermostability of Cassia tora protease inhibitors needs reference.

Caseinolytic assay
1st Ph - Results need statistical analysis.
1st Ph - It is recommended that Aspergillus flavus is written with the full scientific name in the entire document.

Conclusion
1st Ph - Please, make sure that the comment on traditional use is in line with the information in the background section.