Reviewer's report

Title: Evaluation of peptide-rich root extracts of Calliandra portoriscensis (Jacq.) Benth (Mimosaceae) for in vitro antimicrobial activity and brine shrimp lethality

Version: 1 Date: 07 Oct 2019

Reviewer: Sueli Yamada-Ogatta

Reviewer's report:

Dear authors, thank you for the replies.

Although the presentation of the manuscript has improved, there are still some points that need to be changed.

"Solid media growth inhibition assay"

The description of the method is still confusing. What was "autoclaved for 15 mins at 121 oC, allowed to cool at 47 oC and poured into plates" (Page 6, lines 27-28)? Please verify if the description below matches what was done:

The crude and pre-purified peptide extracts of CPr were separated by electrophoresis in SDS-polyacrylamide gels. The gels were cut and deposited into a Petri dish. A single bacterial colony was inoculated into 5 ml TSB and incubated at 37 ºC until it grew up to achieve a cell density equivalent to 0.5 Mc Farland. A 10 μL- aliquot of bacterial cultures was inoculated into 10 mL sloppy agar (0.5% agar) kept at a temperature of 50 ºC. The mixture was homogenized and poured onto the plate. SDS-gel containing markers and buffer were used as negative controls. The 0.1% gentamicin was used as positive control. The plates were incubated at 37 oC during 24 h and the growth inhibition was analyzed.

"Minimum inhibitory concentration determination"

The crude and purified peptides fractions were serially diluted twice? Please verify.

Results

Figures were removed from the manuscript. Please also remove the information related to them in Results section.

Why the authors presented the IC50 and MIC? According to the authors reply, "IC50 is the half maximal inhibitory concentration. What is the relevance of this data?"
Table 3 can be removed.

There is no description of the Plate 1 in the text.

Discussion

The BSLA is not a tool to isolate bioactive compounds form plants, please amend.

Although the Artemia salina assay may also indicate the potential as anticancer compound, in the context of the presented study, I assumed that the assay was to verify the potential toxicity of the extracts, which may indicate the safety of use in animal, including the human. Please verify the description presented in 15-17, page 10.

Conclusion:

In my opinion, conclusion should be limited to "This work has documented the identification…….detected in the plant".

Minor comments

-Please revised "defense";

-Please use CPr throughout the text or C. portoriscensis

-Please correct:

Abstract, line 18: "Artemia salina"

Introduction, page 4, line 9: - "the roots of the plant are"

Page 5, line 2: - "cryodissecation"

Page 6, line 11: "dimethylsulphoxide"

Page 6, line 16: "Manitol"

Page 6, line 27: "Mc Farland"

Page 9, line 28: "validate"

Page 10, line 20: "Artemia salina"

Page 10, line 21: "potential"
Please remove: "In agreement with our finding". You did not evaluate the antiviral activity.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

No

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

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Please indicate the quality of language in the manuscript:

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