Response to the Editor and Reviewer

Ref: molecules-2506648

Title: 'Unveiling the structural characteristics and bioactivities of the polysaccharides extracted from endophytic *Penicillium* sp.'

Journal: *molecules*

Editor-in-Chief

molecules

Thank you for your valuable comments and decision (revision) on my submitted article. Herein, I am submitting the revised manuscript and complete responses to the reviewer's questions. I am thankful to **Editors and Reviewers** for their valuable comments and keen observation.

Editor and Reviewer comments:

Reviewer #1:

The authors of the manuscript undertook the difficult task of obtaining and chemical and biological characterization of intracellular polysaccharides from endophytic *Penicillium radiatolobatum*. Unfortunately, the topic, however interesting and valuable, has not been properly implemented.

Response: We thank you for reviewing our manuscript and making insightful suggestions to improve it. We believe that we have made all the possible changes to the manuscript as per your valuable comments and suggestions, and the revision is highlighted in red color.

1- I regret to say that a significant part of the publication contains information loosely related to the subject of the publication. Reading the manuscript, I had the impression that the authors themselves do not fully know what they would like to convey to the readers and try to fill the word limit by force.

Response: We truly appreciate your detailed review for the improvement of our manuscript. Based on reviewers' comment, we have answered all your queries and evidenced our findings with additional experiments, incorporated those results in this study, compared our work with previous studies, and added further discussion in the revised manuscript.

2- There is also a lack of proper discussion of the results obtained. Here, too, the authors provide data loosely related to the subject of the publication, instead of focusing on an in-depth analysis of their own results in the context of the analysis of other polysaccharides obtained from *Penicillium radiatolobatum*, or if such data is missing, from *Penicillium* sp. An alternative option would the authors could consider is a comparison of their data with results obtained from other fungal polysaccharides of similar structure. The principle is very simple, we compare like with like.

Response: We noted your point and thank you for the suggestions. We have compared the present work with some recent and similar studies and summarized it in Table 2, and appropriate discussion is added in the revised manuscript.

3- I appreciate that the authors of the publication are aware of the need to conduct parallel research on normal and cancer cells in order to check the safety of the tested compounds. Nevertheless, the selection of "normal cells" is incomprehensible to me for two reasons: 1) investigating the impact of compounds on prostate cancer cells, it seems natural to use as a control normal cells from the prostate, e.g. HPrEC (Human Primary Prostate Epithelial Cells) 2) furthermore, the selection of HEK-293 cell line as control was unfortunate because of the fact that these cells were transformed with adenovirus in order to the addition of Ad5 E1A and E1B genes, which immortalized these cells by interfering with cell cycle control and preventing apoptosis.

Response: We agree with you! Actually, for cytotoxicity analysis, we utilized various cancerous cells (PC-3, BT-474, A-549 and Caco-2 cell lines) and non-cancerous cells (HEK-293 and NIH3T3 cell lines). Where tested samples were found non-toxic to non-cancer cells and notably toxic to PC-3 cells (highest activity among above-mentioned cancer cells). Therefore, the HEK-293 cells were used in this study to represent a non-cancerous cell line. However, considering your

comment, the findings of cytotoxicity assay on NIH3T3 cell line have been incorporated in the revised manuscript. Due to the funding crisis at the present moment, we could not test the samples on control normal cells such as HPrEC cells; however, we will include this in a future detailed anti-prostate cancer study of IPS-2.

4- It needs to be also highlighted that the conclusions from biological studies are too far-reaching. Indeed, the authors managed to demonstrate the antioxidant effect of the tested polysaccharides, but in the case of in vitro studies, they only proved a decrease in the viability of prostate cancer cells in response to tested compounds. I cannot agree that pictures from fluorescence microscopy presented in Figure 7 provided the evidence of apoptosis induction, and does not entitle authors to make theories about the mechanism of the observed cancer cell death. I also point out the incorrect use of the terms vitality and proliferation (these are not synonymous).

Response: We truly appreciate your keen observation, and thank you for the comment. Given your concern, we have analyzed the effect of IPS-2 treatments on PC-3 cells and death stages were quantified by reading the cells using flow cytometry. The flow cytometry analysis findings (Figure_9) and related 'Methodology and Discussion' is incorporated in the revised manuscript. Moreover, some incorrect terminologies used in the manuscript have been corrected.

5- I have serious doubts as to the method of determining the IC50 for the performed biological tests, especially after reading the authors' explanation as to the impossibility of determining the IC50 for IPS-1 based on the results of the WST assay conducted in PC-3 cells. "The IC50 of PC-3 cells' viability was defined as 425±8.5 326 µg/mL for IPS-2, but the IC50 value could not be determined for IPS-1 up to the highest 327 tested (1000 µg/mL) concentration (Figure 7a)".

Response: We deeply regret the mistake in writing, and thank you for highlighting the confusing statement. Though, we have re-evaluated the IC_{50} concentrations of the respective experiments, and have amended them in the revised manuscript.

6- Figure captions also require supplementation and detailing. They are too laconic, and statistical significance information is completely incomprehensible.

Response: Thank you for your keen observation and for providing valuable suggestions for improving our manuscript. As you suggested, the Figure captions have been elaborated, and statistical significance has been performed (using one-way and two-way analysis of variance followed by Tukey and Duncan test at $p < 0.05$ significance stage using the GraphPad Prism 8.0 and SPSS program) and incorporated in the revised manuscript.

We believe that we have answered all the queries asked and made all the possible changes to the manuscript as per the reviewers' and editors' suggestions. We are looking forward to the valuable decision on our submission.

Thank you!

Sincerely,

Corresponding author