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OPEN PEER REVIEW

Antifungal Susceptibility of *Aspergillus flavus*, *Aspergillus ochraceus*, and *Fusarium graminearum* to *Ganoderma lucidum* Extract

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About this report

This open peer review (OPR) report shows the public comments made on this article during peer review, by the reviewers, the associate editor and the editor-in-chief, for every revision round, together with the authors' replies. Comments appear only with the consent of the people who wrote them. Reviewers who chose to sign their report are named; the others are shown as "Referee". Comments are edited for language only, and confidential remarks to the editors are not included.

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1 Original submission

3 comments · 29 Jun 2021 – 30 Jun 2021

Referee 1 Reviewer

MAJOR REVISION 29 Jun 2021

Dear author
Please find the comments below:
The inhibition of toxin production by fungi must be investigated.
- Was the extract dried and then used to prepare different concentrations, or were the mentioned concentrations prepared from the filtrate solution?
- The MFC of extracts needs to be determined.
Best Regards

Associate Editor

MAJOR REVISION 29 Jun 2021

Dear editor
This manuscript needs major revision.
Best Regards

Editor-in-Chief

MAJOR REVISION 30 Jun 2021

Dear Authors,
We have received the reviewers' comments on your manuscript; they suggest major changes. Please provide point-by-point answers to the reviewers' comments and highlight them in the revised manuscript. We will reconsider your revised manuscript for possible publication as soon as we receive it.

We are looking forward to receiving your revised manuscript.

Kind regards

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Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Dear Editor,

I would like appreciate the reviewers and editor for the comments and suggestions on our manuscript entitled **"Antifungal Susceptibility of Filamentous Fungi *A. flavus, A. ochraceus,* and *F. graminearum* to *Ganoderma lucidum* Extracts**". The manuscript is revised and all the changes were highlighted letters in the text.

Response to reviewers

Reviewer 1

Comment 1: The manuscript is not presented in an orderly and scientific manner and requires revision before consideration for short communication paper.

Answer to comment 1: The aim of this study was to investigate the antifungal effect of *Ganoderma lucidum* on filamentous fungi important in food industry and has been prepared based on the order of the journal and in line with a scientific manner. This is an original research article and based on the opinions of respected referees, its scientific and technical objections have been reviewed and corrected.

Comment 2: Also, it is recommended that the manuscript should be proof read by a native English editor before resubmission, as there are many statements throughout the manuscript that need clarification.

Answer to comment 2: The manuscript is revised with a native English editor.

Comment 3: In the materials and methods references are needed to be cited

Answer to comment 3: It is done in the manuscript. You can see the changes in title 3.

Comment 4: There are no justification for selecting the concentrations used in the study. What are the reasons for selecting these concentrations.

Answer to comment 4: Based on the studied research papers about antifungal activity of *G. lucidum*, the mentioned concentrations ranged between 0.25-20 mg/ml the reference for these concentrations is as follows (29) that are cited in the article too.

29. Ćilerdžić, J., Vukojević, J., Stajić, M., Stanojković, T., Glamočlija, J. (2014). Biological activity of Ganoderma lucidum basidiocarps cultivated on alternative and commercial substrate. J *Ethnopharmacol* 155(1), 312-319.

Comment 5: Normally, samples should be screened for effectiveness, then the most effective concentration or the ones that are effective are subjected to MIC test (if aqueous extract was not effective, why MIC was performed?).

Answer to comment 5: One of the aims of this study was to compare different solvent extracts for antifungal properties G. lucidum. There was no pre-assumption for applied solvents so the tests were considered as informative data. The concentrations used for the MIC test were reviewed and selected from studies on the similar species or genus of fungi studied in previous research.

So far, no study has been done on the antifungal properties of G. lucidum fruiting bodies and its extracts originated from the northern forests of Iran, and any extract of this species may have such an effect which shows the necessity of conducting this study.

Comment 6: If tests were conducted in triplicates, the standard deviation should have been calculated and presented in the results section.

Answer to comment 6: The standard deviation for fig 3 was already inserted. In fig 3, the standard deviation of the results was showed by error bar for each data paints. In fig 1 and 2 the data was presented as the numerical score of growth inhibition according to CLSI method that is presented as percentage as this order: 0, 25, 50, 75, 100 and the results showed the same percentage.

Each microdilution well is then given a numerical score as follows: (0-no growth; 1-minor growth or around 25% of the growth control; 2-considerable reduction in growth or around 50% of the growth control; 3-minor reduction in growth or around 75% of the growth control; and 4- no reduction in growth was observed (. All tests were repeated 6 times and it was corrected in the text.

Comment 7: The text presented in the results and discussion is not comprehensible, clarification is needed.

Answer to comment 7: Agreed and corrected. Structural were applied in the results and discussion section.

Comment 8: References number 28, 29 and 30 are not cited in text or are not in numerical order.

Answer to comment 8: The mentioned references are sorted by number and there is no more interference in the numbering order.

Comment 9: Conclusion should be limited to the findings of the study only, not previous findings.

Answer to comment 9: Thank you for your opinion, the Conclusion of manuscripts has been revised and edited.

Reviewer 2

Comment 1: The toxin production inhibition of fungi must be investigated.

Answer to comment 1: The aim of this study was to evaluate the antifungal properties of *G. lucidum* and toxin production inhibition was not the objective of this study. Therefore, the mentioned expression about probability of toxin formation inhibition in the abstract and the conclusion was revised and corrected. Given that toxigenic fungi are of great importance in food processing, the possible toxin production property of *G. lucidum* could be investigated in the future. Also, all words related to toxin was removed from the article. To prevent any confusion for referees and other researchers, we preferred to use the word “filamentous” fungi in the title and text instead of the word “toxigenic”. Because all three fungi studied are considered as filamentous fungi and the antifungal susceptibility test (MIC) was performed based on the CLSI protocol M38-A2 for filamentous fungi.

Comment 2: Did the extract dried and then different concentrations were prepared or the mentioned concentrations were prepared from the filtrate solution?

Answer to comment 2: Due to the interference of the solvent effect on the desired concentrations, the extracts are first concentrated and the concentrated extract resolved in DMSO to achieve the desired concentrations. You can see these changes in subtitle 3.4.

Comment 3: The MFC of extracts needs to be determined.

Answer to comment 3: Agreed and revised accordingly. The MFCs were inserted in table 1. The results of MFC were indicated in the table for those fungi that the MIC values were determined for them and the MFC method was also explained in subtitle 3.6.3.

Associate Editor 1

Comment 1: This manuscript needs major revision.
Answer to comment 1: The mistake was corrected in revised version of manuscript. We thank the referees for carefully evaluating our manuscript.

Sincerely

2 Revision 1

2 comments · 5 Oct 2021 – 19 Oct 2021

Batool Sadeghi-Nejad Reviewer

MINOR REVISION 5 Oct 2021

Dear Editor,
1. The title is too long. If possible, change it to: Antifungal Susceptibility of Aspergillus flavus, Aspergillus ochraceus, and Fusarium graminearum against Ganoderma lucidum Extracts.
2. Abbreviations should not be used in the title, so scientific names like Aspergillus and Fusarium should be written in full.
3. In the abstract, scientific names such as Aspergillus and Fusarium should not be abbreviated at first mention and should be written in full.
In line 97: References 28, 29, and 30 are either not cited in the text or not cited in numerical order, and the order of the references in the text is not maintained.
If the mentioned references are sorted by number and there is no further interference with the numbering order, it is best to remove them from the reference list.
4. Line 162: Add to the list of abbreviations: CFU/ml: Colony-Forming Unit.
5. Line 175: 3.6.2 MIC assay, line 177: Correct the sentence to: "First, 100µl RPMI1640 culture medium was added to each well."
6. Lines 188, 268, 276, 277, 325, 335, 336, and 354: Italicize G. lucidum.
7. Lines 208 and 256: Add "the" before "Hydroalcoholic extract."
8. Line 209: After the period, capitalize "In addition."
9. Lines 217, 219, 225, 227, and 230: Add "the" before "aqueous extracts."
10. Line 244: Change "However, the two commercial aqueous extracts" to: "However, both the commercial aqueous extracts and the aqueous extract. . .. . .."
11. Lines 283, 290, and 341: Add "the" before "ethanolic and aqueous extracts."
12. Line 292: Correct the sentence to: "Besides, the commercial standardized aqueous powdered diluted with the culture media (RPMI) was tested in this study."
13. Line 309: Add "the" before "methanolic extract of. . .. . .."
14. Lines 310 and 313: Italicize A. ochraceous.
15. Line 319: Italicize A. flavus.
16. Please ensure that every reference cited in the text is also present in the reference list. References should be adjusted according to the format of the journal, as described below.
Walker DM, Oghumu S, Gupta G, McGwire BS, Drew ME, Satoskar AR. Mechanisms of cellular invasion by intracellular parasites. Cell Mol Life Sci. 2014; 71(7):1245–63. doi: 10.1007/s00018-013-1491-1. [PubMed: 24221133]. [PubMed Central: PMC4107162].

17. Line 209: Table 1. Change "toxigenic fungi" to "filamentous fungi" in the text.

Sincerely

Editor-in-Chief

MINOR REVISION 19 Oct 2021

Dear Authors,
Your revised manuscript was scrutinized, and it still needs some revision. Please revise the manuscript

according to the reviewers' comments and upload the new version with a point-by-point response to the comments.

Yours,
JJNPP EIC

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Revision 2

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Dear Editor,

I would like appreciate the reviewers and editor for the comments and suggestions on our manuscript entitled **"Antifungal Susceptibility of *Aspergillus flavus, Aspergillus ochraceus,* and *Fusarium graminearum* against *Ganoderma lucidum* Extracts**". The manuscript is revised and all the changes were yellow highlighted letters in the text.

Response to reviewer

Reviewer 1

Comment 1: Title is too long. If possible change to: Antifungal Susceptibility of *Aspergillus flavus, Aspergillus ochraceus,* and *Fusarium graminearum* against *Ganoderma lucidum* Extracts.

Answer to comment 1: The title is corrected with respect to your suggestion.

Comment 2: Abbreviations should not be used in the title, so scientific name like *Aspergillus* and *Fusarium* should be written in full.

Answer to comment 2: It is corrected.

Comment 3: In abstract, the abbreviation of scientific names such as *Aspergillus* and *Fusarium* for the first time should not be used for the first time and written in full.
In line 97: References number 28, 29 and 30 are not cited in text or are not in numerical order and the order of the references in the text is not observed.
If the mentioned references are sorted by number and there is no more interference in the numbering order. It is best to remove them from the list of references.

Answer to comment 3: The abstract is corrected, also the references and their numerical order is arranged.

Comment 4: Line 162: Add to abbreviation, CFU/ml: Colony-Forming Unit.

Answer to comment 4: It is corrected. You can see the correction in abbreviation section.

Comment 5: Line 175: 3.6.2 MIC assay, line 177, correct sentence: First, 100µl RPMI1640 culture
medium was added to each well.

Answer to comment 5: It is corrected.

Comment 6: Line 188, 268, 276, 277, 325, 335, 336 and 354: To Write G. lucidum to italic *G. lucidum.*

Answer to comment 6: It is corrected.

Comment 7: Line 208 and 256: Add the before Hydroalcoholic extract.

Answer to comment 7: It is corrected.

Comment 8: Line 209: After point to write capital In addition,

Answer to comment 8: It is corrected.

Comment 9: Lines of 217, 219, 225, 227 and 230: Add the before aqueous extracts.

Answer to comment 9: It is corrected.

Comment 10: Line 244: Correct sentence However, the two commercial aqueous extracts to: However, both the commercial aqueous extracts and the aqueous extract…….

Answer to comment 10: It is corrected.

Comment 11: Line 283, 290 and 341: Add the before ethanolic and aqueous extracts.

Answer to comment 11: It is corrected.

Comment 12: line 292: Correct sentence: Besides, the commercial standardized aqueous powdered diluted with the culture media (RPMI) was tested in this study.

Answer to comment 12: It is corrected.

Comment 13: Line 309: Add the before methanolic extract of……..

Answer to comment 13: It is corrected.

Comment 14: Line 310 and 313: Write italic *A. ochraceus*.
Answer to comment 14: It is corrected.

Comment 15: Line 319: Write italic *A. flavus*.

Answer to comment 15: It is corrected.

Comment 16: Please ensure that every reference cited in the text is also present in the reference list. References should be adjusted according to the format of the journal, as described below. Walker DM, Oghumu S, Gupta G, McGwire BS, Drew ME, Satoskar AR. Mechanisms of cellular invasion by intracellular parasites. Cell Mol Life Sci. 2014; 71(7):1245–63. doi: 10.1007/s00018-013-1491-1. [PubMed: 24221133]. [PubMed Central: PMC4107162].

Answer to comment 16: The references are corrected in adoption with the journal guideline.

Comment 17: Line 209: Table 1. Change "toxigenic fungi to filamentous fungi" in the text.

Answer to comment 17: It is corrected.

3 Revision 2

1 comment · 3 Nov 2021

Editor-in-Chief

ACCEPT 3 Nov 2021

Dear Authors,

It is my pleasure to inform you that your manuscript has been accepted for publication in JJNPP in its current form. We will send it to the publisher for further processing.

Kind regards,
JJNPP EIC