In this study, the Authors reported a new solid-state fermantetaion process of Ganoderma sinense (GS) with Trapa bispinosa (TB) using a response surface methodology, and performed extensive characterization of obtained extracts and purified polysaccharides, in terms of the chemical composition, structure, and biological effects. For the biological studies, the Authors focused on, in particular, on anti-oxidant and pro-/anti-inflammatory effects of the tested materials, by using a validated LPS and H_2O_2 in vitro model with murine macrophages. The work is well-designed, experimental design/rationale well-presented and justified, supported by optimization procedures and dose/time-response experiments. There are concenrs regarding the qPCR data analysis and data presentation style/quality/clarity (please see below Major Comments). The issue raised by the Reviewer also concerns the Introduction and Discussion parts, which do not provide the detailed background and do not refer/ scarcely refer to other previous work.. The Reviewer recommends a minor revision of the English language. The detailed comments and suggesions are listed below.

Major Comments:

1. Title: Please rewrite the title by highlighting the optimized solid-state fermentation process and/or comparison of before and after fermantation, or gighlighting the differences obtained The Authors should more highlight the optimization and the characterization of obtained materials. The analysis of biological activitis was not the biggest and the most extensively studied component in this work.
2. Abstract: please carefully revise and improve the abstract following the order: Background, Aim, Methodology, Results. Please briefly describe the impact of the tested P1 and P2 on the NO production and cytokine secretion. The biological effects are scarcely mentioned.
3. Introduction/Discussion: please describe in detail the anti-tumor, anti-oxidant and anti-/pro-inflammatory effects of Trapa Bispinosa or other herbs reported in previous works, especially focusing on macrophages/immune system if available.
4. Please revise the Materials&Methods and Results section headings. Make them more informative and be consistent with the language style, also revise the English laguage, e.g. 2.3, 2.4, 3.1, 3.2, 3.3, 3.5 (decide for gerund or relative clause in the whole work).
5. Lane 256-258: The description of the qPCR data analysis is enigmatic. How did the Authors analyze the mRNA expression? What did the Authors mean by saying that the expression levels were compared? What house-keeping gene(s) was(were) used, and what method was used to calculate the relative expression? Please revise and correct.
6. A general comment to data presentation: please improve the graphical presentation (quality, size). It is ipossible to read e.g. graph legends, axis labelings. Please revise and rearrange the figures 1, 2,3,4,5.
7. Please revise and improve the language and scientific soundness for all figures and tables captions. Keep the style consistency.
8. Please include the statistical analysis for Fig. 1 and Supplementary Fig. 1.
9. Lane 401: The refering to the results presented in the Fig. 3E should appear earlier in the text. The whole section 3.6. should be placed after the Section 3.3. Alternatively, the whole part of results reporting the biological effects of investigated materials could be placed as last section, and provided with the cytotoxicity analysis transferred from the supplementary part to the main text. Please carefully revise and improve this Results Section.

10. Discussion: Please discuss in detail the obtained results with other works reporting the fermentation process improvement strategies, and the biological activities of obtained materials. These issues were not addressed comprehensively.
a/ Please discuss in detail the changes in inflammatory cytokines, iNOS and NO, compare to other works, and give possible implications/benefits of this biological activity of the obtained materials.
b/ Is there any possible medical use for the obtained extracts? What disease/pathological states? Are anti-oxidant, immuno regulatory properties desirable? Please discuss.

Minor comments:

1. Lane 9: please replace "antioxidative and anti-tumor immune-regulating effects" with "anti-oxidant, anti-tumor, and immunomodulatory effects".
2. Lane 11: please replace "experiment" with "methodology".
3. Lane 16: please replace with "macrophages (RAW 264.7) were".
4. Lane 32: plase replace "anit-oxidation" with "anti-oxidant".
5. Lane 33: please replace "regulation" with "regulatory".
6. Lane 55: please be specific with "condition", e.g. "pathological condition".
7. Lane 62: please be speciic, provide examples for these specific pharmacological activities.
8. Lane 67: please replace "this" with "This process".
9. Lane 68: What drug did the Authors mean? Please be more specific, provide more details.
10. Lane 83:
11. Please replace "Sigma" with "Sigma Aldrich", "DEME" with "DMEM".
12. Lane 87" please replace "Synthase" with "Synthase Activity".
13. Lane 86: please give the cat. no. or bacterial strain origin of the LPS used.
14. Lane 90: please replace "isoplus" with "isolation".
15. Lane 104: The text do not refer to the Table 1 mentioned. Please revise.
16. Lane 114: Please revise the table heading, e.g. Design of Response Surface Experiments.
17. Lane 121: please provide the reference for the method.
18. Lane 122: please replace "20-minute" with "20 minutes".
19. Lane 125, 179, 183, etc.: please replace the centrifugation paameter r/min with xg to easily repeat the centrifugation procedure by others.
20. Lane 127" please replace "20ml" with "20ml of".
21. Lane 160-162: please revise and rewrite the sentence.
22. Lane 165: please rewrite the sentence, e.g. "exhibiting no cytotoxic effects".
23. Lane 169: What did the Authors mean by "drug"? Please be more specific, e.g. the material, extract, etc. No drug was used in this study.
24. Lane 173-175: please rewrite the sentence. Secretion of what?
25. Lane 200: please provide the reference for the Congo red method.
26. Lane 212: please revise the sentence, e.g., replace with "determine the composition of AE1 and AE2 samples".
27. Lane 223-224: please remove "Mass…. As follows:".
28. Lane 234: it should be highlighted that it was mRNA expression analysis, not proteins.
29. Lane 236-239: please rewrite the sentences, e.g., "To investigate the changes in the expression levels of NF-κB pathway-related mRNA, four experimental groups were established: the control group…". Similar comment for Lanes 242-243.

30. Lane 240, 246: please remove “cell solution”.
31. Lane 243: please replace “mRNA” with “genes”, “set up” with “analyzed”.
32. Lane 249: What did the Author mean by “drug”? Please revise and correct as previousley commented.
33. Lane 251: please be more specific with “micro-UV”, revise.
34. Lane 253: please provide the supplier details for the RT kit.
35. Lane 259: Please remove “and discussion”.
36. Lane 326: please rewrite the text regarding MDA, and define MDA here.
37. Lane 329: please replace “MAD” with “MDA”.
38. Lane 336: please rewrite the sentence, e.g. “ “…alcoholic extracts was performed”.
39. Lane 351: please rewrite the sentence, e.g. “The content distribution of fractions with different weight-average molecular masses in P1 was relatively homogeneous (Table 3).
40. Lane 365: please replace “image” with “images”.
41. Lane 395 and others: please write the name of compounds, metabilites with lower case letter.
42. Lane 396, 397: please improve the scientific language, revise “saw”, “experienced”.
43. Lane 437: please replae “functions” with “properties” or “activities”.
44. Lane 452, 469: evaluation criteria for what/to assess what? Please be more specific.
45. Lane 461: please define the abbreviation “T-AOC”.
46. Lane 485: please revplace “in biological” with “in the biological”.
47. Lane 244 and others to 412: please define “VC”. Include the term in the Materials part and Figure captions to avoid confusion about the group treatment design.
48. The whole manuscript: please revise the terms pre-fermentation and before fermentation; alcohol and alcoholic extracts, they are used interchangeably. Please revise and decide for one style to avoid confusion.
49. Supplementary File: Table S6; Please revise “Contencontent” in the Lane 1 of the Table.
50. Supplementary File: Table S6; please revise the “Compound” column (revise column widths), some names are split into two lanes resulting in uneven text position, e.g. Ethyl 4-hydroxybenzoate, 4'-Prenyloxyresveratrol, 3',4'-Anhydrovinblastine, 1,3,6-Tri-O-galloyl-β-D-glucose, etc.