Rebuttal: Identification of bioactive compounds in phenolic rich extract from marine red algae Gracillaria edulis (Gmelin) Silva to combat obesity and type-2 diabetes

Reviewer # 1

We thank the reviewer 1 for his/her helpful comments, which helped us to improve our manuscript immensly.

In this manuscript, entitled “Identification of bioactive compounds in phenolic rich extract from marine red algae Gracillaria edulis (Gmelin) Silva to combat obesity and type-2 diabetes” by Gunathilaka et al., the authors investigated the anti-oxidant and anti-diabetic effects of methanol extract and its fractions from red algae Gracillaria edulis (G. edulis) in-vitro. They found the ethyl acetate fraction might be the most potential source in all fractions and they identified six compounds from this fraction using GC-MS. Overall, this study is fine and worthy of publication in this journal. However, here are some questions or suggestions for the authors in order to validate their findings:

1. Since all the experiments were in vitro and it is difficult to find any experimental design related to obesity, the title of the manuscript is very inappropriate.

   We thank for the comment made and as suggested the title has been changed as given below

   Identification of bioactive compounds in phenol-rich extract from the marine red algae Gracilaria edulis (Gmelin) Silva to combat Type-2 diabetes.

2. The authors did find 6 compounds in EA fraction showed in table 4, however, are they all phenolic compounds? Why did the authors emphasize “bioactive compounds in phenolic rich extract”?

   Here, in the methodology, we have separated the polysaccharide portion using 70% ethanol and the remaining polyphenol portion was used for the analysis. That’s why title indicates as “bioactive compounds in phenolic rich extract”.

3. The authors mentioned that the inhibitory activity of acarbose or the reference drug was considered as 100% for glucose diffusion inhibitory activity assay (lines 160-161, p. 5). However, the unit the authors used is glucose concentration (μg/mL).

   We thank the reviewer for his or her comments but, we measured the inhibitory activity of the sample on glucose diffusion through a dialysis membrane and it is measured by the glucose concentration in the external solution (μg/ml). If the glucose concentration in the external solution is high, the absorption is less. Therefore, the inhibitory activity of the sample on glucose diffusion is less. As the acarbose is a standard anti-diabetic drug, here we consider the inhibition by acarbose (standard) on glucose diffusion is 100%. This means the glucose concentration in the external solution become low.

4. The authors mentioned that amongst the tested fractions, ethyl acetate fractions exhibited maximum inhibition of glucose diffusion at 180 min and glucose concentration of the external solution was found to be 38.15±1.11μg/mL (lines 162-164, p. 5). However, the fraction showed highest inhibition of glucose diffusion at 180 min is chloroform fraction showed in figure 3.

   Thank for pointing out the mistake. There is a small mistake in the graph while handling the data set. What you mentioned here is right. The graph shows highest inhibitory activity from chloroform fraction, which is not correct. When you look at the Table A1, it showed the highest inhibitory activity by an ethyl acetate fraction at 180 minutes (38.15±1.11μg/ml). Corrected the graph.
5. The authors indicated that the highest DPPH radical scavenging activity was observed in ethyl acetate fraction ($IC_{50}$: 3.19±0.02 mg/mL) and crude methanol extract ($IC_{50}$: 3.19±0.02 mg/mL) compared to the standard Trolox ($IC_{50}$: 0.011 mg/mL). However, the $IC_{50}$ of EA fraction showed in table 2 is 3.17±0.04.

Thank you again for pointing out the mistake. This is also a typing mistake. The highest DPPH radical scavenging activity was reported in ethyl acetate fraction with $IC_{50}$ of 3.17±0.04 mg/mL. The value indicated in the paragraph is not correct which is $IC_{50}$ value of crude methanol extract (3.19±0.02 mg/ml).