Author's response to reviews

Title: In vitro inhibitory effects of plant-based foods and their combinations on intestinal alpha-glucosidase and pancreatic alpha-amylase

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Author's response to reviews: see over
Dear Editor

I, along with my coauthors, would like to ask you to consider the attached manuscript entitled “In vitro inhibitory effects of plant-based foods and their combinations on intestinal $\alpha$-glucosidase and pancreatic $\alpha$-amylase” for publication in BMC Complementary and Alternative Medicine as an original article. In accordance with your suggestions, the manuscript has been revised for English, and the answers have been shown below this section.

Reviewer's report:

Title:
1. “water extract” should replace “extract”.
   Re: We used the dried extract.

2. better to mention the form of the plants (dried).
   Re: The form of plant was referred in the Method/Abstract.

Methods:
3. Should refer to table 1 in this section.
   Re: Table 1 was referred in Collect and Extraction part in line 111.

4. Should indicate if plants were authenticated.
   Re: The plants do not authenticate because they are a well known plant that used for human diet.

5. Should give reference(s) to the method(s) of the water extraction and drying of the plants.
   Re: The reference of the extraction method was added to the section in line 116.

6. Should indicate in this section or in the other sections what plant components are expected to be in the studied water extract and/or wither the water extract is the one usually consumed.
   Re: According to the reviewer suggestion, the statement was added in the introduction in line 101-103.
7. Should indicate the type/model of grinder, spray drier and spectrophotometer used, particle size of the ground (milled) parts and temperatures of drying.
   Re: The model of spray dryer and spectrophotometer were added and the condition of spray drying process was clarified to the section in the line 121.

8. The ratio of the weight of plant material to water used in extraction need to be mentioned.
   Re: The weight of plant material and the volume of water for the extraction were added to the section in Line 119.

9. There is a need for further details or clarification of the methods used for the determination of phenols and flavonoids contents and enzymes inhibitory activity with respect to samples weights and aliquots used.
   Re: The weight of sample and volume for making the solution was added to the section in line 127-128 (polyphenol), 136-137 (flavonoid), 146-147 (glucosidase), and 163-164 (amylase).

10. It is not clear whether the determination of phenols and flavonoids was done on the dried- or the aqueous extract.
    Re: We modified the statement in line 127-128 (polyphenol), 136-137 (flavonoid).

11. The combinations of extracts do not show the amounts used from each dried extract.
    Re: The detail of combination regarding the amount of each dried was added in the line 177-178.

12. In the calculation of inhibitory activity, the “control” and “sample” in the formula used should be explained.
    Re: The detail of formula was explained in the line 157-158 and 173-174.

Results:

13. Should check the numbering of figures in the text as well as in the list.
    Re: We have already checked and changed them all.

14. No reference is made to Table 1 in the text.
    Re: Table 1 was cited in the text in line 189.
15. The last two parts dealing with enzymes inhibitory activity and IC50 require more details or clarifications.
   **Re:** The detail of IC50 calculation was indicated in the data analysis.

16. Better to change Figures into table form at least for Figure 1, it is much easier to obtain the information.
   **Re:** We changed the figure 1 to table 1.

17. Better to have the plant in the x-axis and the % inhibition in the y-axis with a legend for figures 3-5 if space is not enough for the plant name.
   **Re:** We did not change the figures.

18. Concentrations of extracts should appear on the figures/table.
   **Re:** The concentration of extracts was added to the figure legends.

19. In page 20 Line 4 from below: was significantly increased by …” is not correct and requires rewriting e.g. “to” should replace “by”.
   **Re:** The sentence was modified according to the comment.

**Discussion**

20. Any explanation or mode of action for the synergistic enzyme inhibition of roselle combinations?
   **Re:** The explanation for synergistic effect was added to the section in line 310-322.

21. Indicate the importance of the high inhibitory effect of mulberry leaves extraction glucosidase as compared to the effect on amylase.
   **Re:** The statement was added to the section in line 275-283.

22. Based on the results of the study, what plants are recommended to be taken for the prevention of diabetes and at what level?
   **Re:** The statement was added to the section in line 325-329.
Reviewer's report:

1. Usually flower contains flavonoids or isoflavonoids, so that it is not strange that Roselle, Chrysanthemum and Butterfly pea contain flavonoids or isoflavonoids. Those flowers should have the inhibitory effects.
   **Re:** According to the comment, it is exactly true that edible plants contain polyphenol and flavonoids however, the content of phytochemical in each plant are difference and also may show the different potency.

2. Flavonoid is a kind of polyphenol as well.
   **Re:** Yes. However, there are other types of polyphenol that also contain in the extracts such as gallic acid.

3. AlCl₃ can detect only 5-hydroxy flavonoid, and sodium acetate can detect only 7-hydroxy flavonoid by UV bathochromic shift. How do you think about the existence of the other type of flavonoids?
   **Re:** This assay is commonly used for the estimation of flavonoid content that many research articles are cited. For total flavonoid determination, it needs to use HPLC.

4. Those expected polyphenol should be suspected to EGCG, ECG, or hydrolysable tannin. What is the reason for selecting gallic acid as a reference, even though the other type of polyphenol is expected?
   **Re:** We do not expect EGCG, ECG, or tannin as a major polyphenol in the plants. For example, roselle and butterfly peas mainly contain anthocyanins. The colorimetric assay based on the reaction of Folin-Ciocalteu reagent (FCR) is a method widely used for the determination of total phenols in plants. Most of standard recommend to use gallic acid for a standard.

5. How do you confirm these Food materials, and by whom?
   **Re:** The plants do not authenticate because they are a well known plant that used for human diet.

6. What is the profit of plant-based foods, even though we have effective acarbose, voglibose. I don't think the plant-based foods are more effective than these drugs.
Re: Acarbose and voglibose are a drug used for treatment of diabetes. Although the inhibitory activity of plant-based foods may be less active than the drug but they are considered safe for human consumption. We consume these plants as a daily food which can help us to prevent the rise of postprandial glycemia. This will have a beneficial effect for prevention of diabetes Moreover, they are quit safe and less side effects when they compare to the drug.

7. You say that “many scientists have realized ---“, please refered about that. It must be cleared the reason for their combined inhibitory effects. This article should rewrite the analysis of acquired results (Table and Figures).

Re: We changed the sentence “many scientists have realized” to “many scientists have investigated”. We also rewrote some sentence of the analysis of the results.

7. In the result section Line 2 from the above. Figure 2 must be Figure 1.

Re: We have done a re-edit of the figure number.