Author's response to reviews

Title: Alpha-Glucosidase Inhibitory Activity and Antioxidant Activity of Flavonoids Compound and Triterpenoid Compound from Agrimonia Pilosa Ledeb.

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Author's response to reviews: see over
Dear Editor,

We are highly appreciated for these helpful reviews from you and the reviewers about our manuscript entitled “α-Glucosidase Inhibitory Activity and Antioxidant Activity of Flavonoids Compound and Triterpenoid Compound from Agrimonia Pilosa Ledeb.” (MS: 4023323951046452). We have revised carefully for whole manuscript according to reviewers’ comments. The major revised portions and response to reviewers’ comments were listed below. We hope that the revised manuscript is acceptable for publication.

Thank you very much for your continued attention.

Best wishes,

Liancai Zhu

Response to reviewers’ comments:

Reviewers' comments:

Reviewer #1: Major Compulsory Revisions

1. Additional experiments should be done in compounds analysis.

Answer: The compounds in TC and FC have been analyzed by using HPLC. In FC, 10 flavonoids were identified and quantified (See Table 1 and Figure 1) and 6 triterpenoids were identified and quantified in TC (See Table 2 and Figure 2).

2. There are no correlation statics, so the conclusion is uncorrect.

Answer: When we study the bioactivities of one compound, we only need to test the activities of the compound at different concentrations and the correlative analysis isn’t essential.

3. The total flavonoids content in FC is 316.53±6.37 mg/g by using the aluminum chloride colorimetric method with quercetin as a standard. And the total triterpenoid content in TC is 415.97 ± 5.15 mg/g with Ursolic as a standard. What other components are? So, the author could not conclude that flavonoids and triterpenoid responde for that bioactivities.

Answer: In this paper, we put forward that the bioactivities is attributed to the FC and TC. FC consists of some flavonoids and other ingredients, and TC consists of some
triterpenoids and other ingredients. So we think that FC or TC as a whole, not just flavonoids or triterpenoids, are responsible for the good clinical effects of APL on T2MD through targeting oxidative stress and postprandial hyperglycaemia.

Reviewer #2:
1. Many minor errors are revised in PDF, please check and revise.
Answer: Some wrong English grammars have been modified, and some incorrect expressions have been corrected in the relevant places.

2. Time collected plant sample and identifier should be given.
Answer: The dried entire plants of APL were purchased from Western Medicine City (Chongqing, China) in 2011 and verified by Changhua Wang (Chongqing Academy of Chinese Materia Medica, China). We have added these contents in the section “Plant materials and preparation of the extract”.

3. Reference “Antioxidant Activities of Aqueous Extract from Agrimonia pilosa Ledeb and Its Fractions” was reported by author in Chemistry and Biodiversity, 2009. which is the difference between both works?
Answer: The differences between both works are as follows:
(1) Their subjects are different. The reference article described the aqueous extract (AE) from Agrimonia pilosa Ledeb were partitioned successively by petroleum ether, AcOEt, and BuOH to the corresponding soluble fractions. The subjects were AE and its fractions. In this paper, we separated and enriched flavonoids compound (FC) and triterpenoid compound (TC) from Agrimonia pilosa Ledeb by using liquid chromatogram as subjects.
(2) Their contents are different. In the reference article, we only studied the antioxidant activities of AE and its fractions. In this paper, we played emphasis on the $\alpha$-glucosidase inhibitory activity, which was reported for the first time. Our study clarified that the FC and the TC could be responsible for the good clinical effects of APL on T2MD through targeting oxidative stress and postprandial hyperglycaemia.

4. The method for FC and TC collected is difficult to understand. How to determine that the method can collect all or partly FC and TC?
Answer: (1) We have drawn a diagram to clarify the collected procedure of FC and TC, please see Figure 1. (2) In the entire collection process, thin layer chromatography control with 10% sulfate in ethanol as color-developing agent was adopted in order to collect all flavonoids compounds and triterpenoid compounds.

5. Phytochemistry for this plant is abundance in reference, especially triterpenoids and flavonoids, compounds in TC and FC should be analyzed using HPLC or HPLC-MS. It is essential for publication. when compounds are determined in FC and TC, activities for antioxidant and α-glucosidase inhibitory activities should be discussed to elucidate the clinical effects of Agrimonia pilosa.

Answer: The monomeric compounds in TC and FC have been analyzed by using HPLC. In FC, 10 flavonoids were identified and quantified (See Table 1 and Figure 2) and 6 triterpenoids were identified and quantified in TC (See Table 2 and Figure 3). And the activities of these quantified flavonoids or triterpenoids have been discussed.

Reviewer #3
In my opinión this manuscript needs a major compulsory revisión. The sections "Methods" and "Results and Discussion" should be rewritten, because there are a lot of inaccuracies, particularly in relation to the fractionation process, isolation and thin layer chromatographic identification of fractions FC and TC (?). The manuscript does not explain how to prepare the stock solutions for use in various tests. Likewise, it is not explained what it is and what implications mixtures FC: TC 4:1, 1:1 and 1:4. In Figures, the results are presented as data outside of the scale level, or simply in a distorted scale. In my opinion, this article should be re-written and must be resubmitted for review. It is not publishable without further major review.

Answer:
(1) The sections "Methods" and "Results and Discussion" has been checked and revised. In this paper, the fractionation was NOT used. In addition, the isolation process has been rewritten and a diagram has been drawn as Figure 1 for clarifying the collection procedure of FC and TC.
(2) The preparations of test samples including solvent and concentration have been clarified in the section "Methods".
(3) “mixtures FC: TC 4:1, 1:1 and 1:4” has been revised as three complexes with
mass ratio of FC and TC as 4:1, 1:1 and 1:4.

(4) All the figures have been re-drawn in order to avoid data outside of the scale level.