Author’s response to reviews

Title: Analysis of Molecular Networks and Targets Mining of Chinese Herbal Medicines on Antiaging

Authors:
Qi-Yu Jiang (514720986@qq.com)
xiao-sheng SUN (sunxiaosheng@gzucm.edu.cn)
mei-si ZHENG (2036007516@qq.com)

Version: 1 Date: 07 Oct 2016

Author’s response to reviews:
point-by-point response letter

Reviewer #1:
(1) The language of the manuscript needs extensive revision.

Response: we have improved the grammars and expressions of the manuscript, and lots of sentences are rewritten.

(2) the manuscript does not include any reports/observations related to hepatotoxicity or renal toxicity of these medicines.

Response: In the revision manuscript, hepatorenal toxicities of all herbal medicines have been checked and add a column in table 1 to describe hepatorenal toxicities.

The added sentences in “Results” section( line 12, page 5)are as follows:

The hepatorenal toxicities of all collected herbal medicines are checked in “Toxicologied of Chinese Medicines”[21]. Most of collected herbal medicines have no hepatorenal toxicitiy and are safe for human body except for Bu gu zhi(Fructus Psoraleae) and He shou wu(Fallopia
multiflora). Bu gu zhi can cause kidney disease when using for a long time[21]. He shou wu can cause liver damage when using for a long time[21].

(3) The discussion of the manuscript is too elaborate. It must be rewritten in such a way as to discuss the key findings of the present study in comparison with earlier studies. As many recent references as possible should be included in the manuscript.

Response: In the revision manuscript, the “Discussion” section has been extensive revised to highlight the findings of this study compared with existing research. The revised sentences are in “Discussion” section(beginning at line 23, page 8). Recent references have also been added ([26]-[30]). There are two points of discoveries in this study:

1. Many existing studies have researched on anti-aging mechanisms of herbal medicines at target genes level. However, most of these studies focus on single or some individual target genes. Compared with existing researches, in this study, important targets, target pairs and regulatory networks of kidney-tonifying herbal medicines related to anti-aging have been found by data mining. Target pairs and regulatory networks of targets can reflect more potential interactions among targets and comprehensive effects on anti-aging.

2. At present, a large number of anti-aging related evidence of kidney-tonifying herbal medicines mainly focuses on the two factors: (1) Improving antioxidant capacity and reduction of free radical. (2) Influences on cell apoptosis. However, this study found that kidney-tonifying herbal medicines may achieve anti-aging in multi pathways.

Reviewer #2:

(1) The authors need to use more systematic selection criteria in collecting 'tonifying-kidney' herbes and main ingredients. They confirmed the effect of anti-aging by reading literature either in PubMed or Chinese biomedical literature database (CBM), but none of the detailed criteria were disclosed. I believe the specific selection criteria of being effective against aging need to be fully disclosed. I also wonder how many authors involved in literature review. Typically, people would use a majority-voting strategy, where at least 2 or 3 experts review the literature
independently (in this case to assess the effect of anti-aging effects of herbes or ingredients) and then combine their results. This approach will be less biased than a single-person review.

Response: In the revision manuscript, we have added the details of reading literature either in PubMed and Chinese biomedical literature database (CBM). The added sentences in “Data preparation” section (line 30, page 3) are as follows:

When in the above process, Pubmed and CBM databases were retrieved by two researchers independently. Only the consistent opinions of them are collected as results.

(2) In general, more background information is needed. The study uses two groups of herbal medicines: tonifying-kidney yang and tonifying-kidney yin. However, no rationale is given for grouping these two separately. I don't think all the readers of the journal would just know the difference between 'yang' and 'yin'. What are they and why should they be separated?

Response: In the revision manuscript, we have added the details and introductions of kidney yang – tonifying and kidney yin- tonifying. The added sentences in “Background” section (line 7, line 13, page 2) are as follows:

kidney yin is the source of the yin qi of human body, which can nourish and moisten the organs, tissues, muscles, etc. And kidney yang is the foundation of the yang qi of human body, which has the function of warming and promoting.

According to “Chinese pharmacy”, the herbal medicines which channel tropism attributes include kidney channel in “deficiency-tonifying” section are kidney-tonifying medicines. And the kidney-tonifying medicines in “Yin-tonifying” section are kidney Yin-tonifying medicines, the kidney-tonifying medicines in “Yang-tonifying” section are kidney Yang-tonifying medicines.

(3) The authors uses -log(P) as a means to indicate different levels of importance (significance) of biological functions or pathways. However, using the p-value alone in assessing potential importance is very risky, especially when comparing the results across multiple data sets of different sizes. For example, "All the signaling in tonifying-kidney-Yin herbal medicines are
more significant than tonifying-kidney-Yang herbal medicines”. (Page 8 Lines 10-12). The authors claim that these biological functions/pathways are more significant in one group than the other at many other parts of the manuscript based on P-value alone. The bigger size of the Yin group might have affected the p-values.

Response: In bioinformatics, the p values of the enrichment analysis are calculated by Fisher's test (A statistical method). It is a recognized evaluation standard. The p values are automatically calculated by analytical tools. This is a generally accepted research method in bioinformatics. So using the p values to evaluate results is reliable. In the revision manuscript, The added sentences in “Mining analysis of common target genes” section (line 1, page 5) are as follows:

In bioinformatics, the p values of the enrichment analysis are calculated by Fisher's test. It is a recognized evaluation standard.

(4) In one of the authors' recent study published at the Current Bioinformatics last year (reference #3 in the current manuscript), they did a fairly similar study on herbal medicines with anti-aging effect. I guess the set of herbes are comparable between the two studies and the results deserve to be compared and discussed.

Response: The published study only uses some of the sample data to test the feasibility of the gene target mining algorithm, focusing on the introduction of new mining algorithm for targets. However, this study is the biological analysis of a large number of target data, focusing on the analysis of associations of anti aging related targets and regulatory networks of targets.

(5) There is no discussion of limitations of the study.

Response: In the revision manuscript, we have added the limitations of this study. The added sentences in “Discussion” section (line 31, page 10) are as follows:

This study gives the anti-aging mechanisms of Kidney-tonifying herbal medicines, and discovers associations and regulatory networks among targets. However, it is only based on the existing known chemical compositions and targets. In the future, more unknown ingredients and targets
will be found, and the research results will be richer than now with the analysis methods proposed in this study.

(6) There are numerous grammatical errors and awkward sentences, which make the manuscript hard to read. The manuscript needs a thorough revision by a native English speaker with enough experiences in scientific writing. I highly recommend the authors to use a professional editing service.

Response: we have improved the grammars and expressions of the manuscript, and lots of sentences are rewritten.

(7) The current study relies on the main ingredients of the selected 28 Chinese herbal medicines. I think the most important thing in herbe-based oriental medicines compared to the western medicines is that not only the main ingredient(s), but also various other ingredients in the herbes work together to exert the final effect. I wonder if this point needs to be discussed in the context of the current analysis focusing on the main ingredient.

Response: This study focuses on the main ingredients. Compared with western medicine, the combination of Chinese medicines work together may have a combination effect. So in this study, the data mining on the associations of targets of the main ingredients can reflect combination effects of Chinese medicines. In the further study in the future, we will consider other ingredients in analysis.

(8') kidney-tonifying' would a better alternative to 'tonifying-kidney'.

Response: In the revision manuscript, we have corrected this expression.

(9) The complete list of collected 28 herbal medicines, a complete version of Table 1, should be provided.
Response: There are many ingredients and many targets in each herbal medicine. A complete version of Table 1 in the revision manuscript may be too large, the detailed original data (including ingredients and targets) of collected 28 herbal medicines have been provided in attachments (supplementary material) in the revision manuscript.

(10) The authors included 28 supplementary files, none of which were referenced in the main text. Majority of them were simply output files of Bingo tool. Other files such as "all collected target genes.xls" and "mining results of tonifying-kindey-yang/yin-.xls" would need more details (captions).

Response: Most of supplementary files are output files of “Bingo” in order to show reliability of data analysis processes. "all collected target genes.xls" is a file that shows all collected target genes of 28 herbal medicines. "mining results of tonifying-kindey-yang.xls” is a file that shows that the base association data outputted by mining algorithms for all collected target genes of tonifying-kindey-yang herbal medicines. "mining results of tonifying-kindey-yin.xls” is a file that shows that the base association data outputted by mining algorithms for all collected target genes of tonifying-kindey-yin herbal medicines.

(11) In Abstract: "About 28 tonifying-kidney herbal medicines~". The term 'about' is not necessary, if the total number of collected herbal medicines is exactly 28.

Response: In the revision manuscript, we have corrected this expression.

(12)Some of the key references such as [1] are not available online for review, and they seem to be in Chinese. I would encourage the authors to use references in English as much as possible for non-Chinese readers.

Response: In the revision manuscript, we have added more references in English. However, lots of studies of Chinese medicines published in Chinese literatures.

(13)Page 7 Line 29: "The threshold of -log(p) value in this study is 0.05." I believe 0.05 was the threshold for p-value.
Response: In the revision manuscript, we have corrected this expression.

(14) Some part of the result sections would be more appropriate if they are in figure legends. Examples would be Page 7 describing figure x-/y-axis.

Response: The figures are generated by the analysis tool automatically according to the data. We can artificially redraw it according to the data and add the figure legends, but it would be very demanding.

(15) I don't see the point of Figure 5. A summary table would be much better.

Response: Figure 5 is the pathway enrichment analysis of the targets of tonifying-kindey-yang herbal medicines, to show targets of tonifying-kindey-yang herbal medicines are mainly enriched in which biological pathways. The enrichment degree of various pathways can be displayed and compared more intuitively by figure 5 than by a table.

(16) IPA includes enrichment analysis in terms of GO and KEGG in their Core analysis. I wonder why the authors had to use Bingo, a Cytoscape plugin, to do the enrichment analysis.

Response: Cytoscape is open-source software for integration, visualization and analysis of biological networks. “Bingo” is a plugin that widely used to do functional enrichment analysis, clustering analysis, and network comparisons in the field of bioinformatics. This study uses “Bingo” to do GO enrichment analysis. “Bingo” can connect to the KEGG database. In the revision manuscript, we have added descriptions about it. The added sentences in “Mining analysis of common target genes” section( line 37 ,page 4).

(17) Page 9 Line 4: "the maximum P value". -> It's the lowest (minimum) P value.
Response: "the maximum P value" would be the correct expression, the sentence “the maximum P value is also less than 1.20E-05” in the context means that all the chosen P values are less than 1.20E-05, so the maximum P value in all the chosen P values less than 1.20E-05.

(18) Many terms used in the manuscript require some explanations or definition. I guess this is partially because the authors just took outputs from IPA, Bingo, and their gene-mining program. Examples include "contribution value" (Page 6 Line 51), "Ratio" (Table 3 on Page 8), 'association degree' (Page 9 Line 10), and etc.

Response: the sentence “score” in table 2 denotes contribution values of target genes involved in.” in the context means that degree of gene involvement. "Ratio" means proportions. “association degree” is a parameter outputted by mining algorithm, it shows the degree related to frequency in all data.

(19) I wonder why the authors limited their search for important targets to just 'pairs of targets'. Since the current approach is a network-based one, they can use a sub-network instead.

Response: 'pairs of targets' show the base association between targets. And the network is built based on data of association targets. This is a step by step process in this study, so 'pairs of targets' is important. The built networks also are results of this study.

(20) The values on the edge (association score?) make the network image complicated in Figures 9 and 10 (particularly Figure 9). Instead of showing all the values, I recommend the author to represent these values such as thickness of edge and etc.

Response: the values can give more details of the associations between targets.

(21) I am not sure about the journal's policy regarding acknowledging other peoples, but in general, the authors need to be specifically say whom they are acknowledging (their names and affiliations). The manuscript currently has 'We acknowledge all the partners who participated in this research and offered fruitful suggestions to carry out this study.'
Response: “We acknowledge all the partners who participated in this research and offered fruitful suggestions to carry out this study” means that acknowledge the three authors of this study. Because their names and affiliations have been written in title page, so the information is ignore here.

(22) The "Availability of data and materials” section indicates that the generated datasets are available in the HIT database (http://lifecenter.sgst.cn/hit/), but I couldn't find any specific datasets related to this study from this database. It would be better if the authors prepare a separate page for this study or provide the datasets (28 herbes, their targets, and etc) as supplementary materials.

Response: I would like to express the target data can be found in the HIT database. The detailed original data (including ingredients and targets) of collected 28 herbal medicines have been provided in attachments (supplementary material) in the revision manuscript.