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Establishment of an HPLC Fingerprint and Cluster Analysis for Miao Ethnic Medicine *Osbeckia opipara*

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Contents

1 **Original submission** Hadi Ghanbari, Associate Editor 2
2 **Revision 1** Hadi Ghanbari, Associate Editor 3
Authors' reply to reviewers 4
Reviewer and editor comments 9

1 Original submission

2 comments · 22 Mar 2024 – 30 Apr 2024

Hadi Ghanbari Reviewer

MAJOR REVISION 22 Mar 2024

Dear authors:
Please find my comments file.

Associate Editor

MAJOR REVISION 30 Apr 2024

Major revision

2

Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Reply to Reviewers

Manuscript ID: 146396

Manuscript Title:Establishment of HPLC Fingerprinting and Cluster Analysis for Miao Ethnic Medicine Osbeckia opipara

Reviewer 1:

1. Please provide a concise overview of Miao medicine in two lines, mentioning the practitioner Zhengshi Wu only once (Introduction). Omit his name and specific descriptions from other sections of the manuscript. In the abstract, emphasize the significance of the species in traditional Chinese medicine and Miao medicine instead.

Response:Thank you very much for your careful and rigorous review. I have provided a brief overview of Miao medicine as requested and included it in the second paragraph of the introduction section of the manuscript.

2. Kindly include the herbarium code of the species.

Response:Thank you very much for your suggestion. The code has been provided and added to the manuscript.The voucher specimen (20200816) was archived at the Herbarium of Guizhou University of Traditional Chinese Medicine.

3. It is important to detail the data analysis process for the similarity study. For instance, specify which data is utilized, such as retention time of main peaks or peak area as well as other details.

Response:Thank you very much for your suggestion. We have added the above questions and cited references in the article.

the chromatograms of the 11 batches of O. opipara test samples were matched with the reference fingerprint chromatogram. Similarity evaluation was conducted by comparing the peak areas through correlation coefficient similarity, a method is derived from the Chinese Pharmacopoeia.

Reference sources:Shen, M.R., He, Y., and Shi, S.M. (2021). Development of chromatographic technologies for the quality control of Traditional Chinese Medicine in the Chinese Pharmacopoeia. J Pharm Anal 11, 155-162. 10.1016/j.jpha.2020.11.008.

4. Provide a precise description of how the common peaks are selected (13 peaks), supported by references.

Response:Thank you very much for your constructive review comments. We have added this suggestion to the text and highlighted it in red.

To establish the characteristic and comprehensive fingerprint, 11 batches of the *O. opipara* samples from different origins were analyzed by HPLC and standardized by the Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine (Version 2004A). There are 13 common peaks in the HPLC fingerprints with good resolution, which was regarded as the reference chromatogram (Fig. 8). Among these 13 common peaks, peak 8 was chosen as the reference peak (S) due to its high content, high intensity and moderate retention time in the *O. opipara* chromatograms. The relative retention time (RRT) and relative peak area (RPA) with respect to the reference peak were then measured. The RRT and RPA statistic in HPLC fingerprints was useful for the quality assessment of *O. opipara*.

Reference sources:Guo, H., Zhang, Z., Yao, Y., Liu, J., Chang, R., Liu, Z., Hao, H., Huang, T., Wen, J., and Zhou, T. (2018). A new strategy for statistical analysis-based fingerprint establishment: Application to quality assessment of Semen sojae praeparatum. Food Chem 258, 189-198. 10.1016/j.foodchem.2018.03.067.

5. Please conduct a thorough review of the manuscript to correct any grammatical errors.

Response: Thank you very much for your insightful suggestion.The manuscript has been edited by a native English editor.

Reviewer 2:

1. What is the meaning of the “similarity of 11 batches of Osbeckia opipara medicinal materials”? Is the area under the peak of 13 similar peaks compared, or is it only sufficient to compare the number of these peaks? Mention this in the text. (table 6)

Response:Thank you very much for your suggestion. We have added the above questions and cited references in the article.

The chromatograms of 11 batches of *O. opipara* test samples were matched with the reference fingerprint chromatogram. The similarity evaluation was conducted by comparing the peak areas through correlation coefficient similarity(This method is derived from the Chinese Pharmacopoeia).

Reference sources:Shen, M.R., He, Y., and Shi, S.M. (2021). Development of chromatographic technologies for the quality control of Traditional Chinese Medicine in the Chinese Pharmacopoeia. J Pharm Anal 11, 155-162. 10.1016/j.jpha.2020.11.008.

2. Please explain sentence " it can be seen that when the classification distance is 25 ". How is this cluster drawn?

Response:Thank you very much for your careful review. We have reinterpreted this clustering diagram and the following is the explanation and drawing of the clustering diagram. And revised and supplemented in the text.

Using the peak areas of common peaks from 11 samples of *O. opipara* as variables, a systematic cluster analysis was performed using SPSS 26.0 analysis software. The between-groups linkage method was employed, with Euclidean distance as the measure for clustering.At a distance of 25, all objects form a single large category without any further division, indicating that there are relatively small differences between medicinal herbs from different origins in *O. opipara*.

3. It is better to start the abstract by mentioning the medicinal importance of the Osbeckia opipara plant and then state the purpose of the work.

Response:Thank you for this important point. Rewrite the first part of the abstract as follows.

Osbeckia opipara,a traditional Miao medicine,is commonly used by the renowned national-level Chinese Traditional Medicine practitioner Zhengshi Wu for clinical treatment of diarrhea,noted for its strong antioxidative and anti-inflammatory antidiarrheal effects.This study aims to establish a High-Performance Liquid Chromatography (HPLC) fingerprint for *O. opipara*.The development of this fingerprint will provide new evidence and technical means for the scientific evaluation and effective quality control of *O. opipara*.

4. I didn't understand how the correlation coefficient in table 4 and 5 was calculated and what variables were involved in its calculation.

Response: Thank you for your question.The precision, repeatability, and relative standard deviation (RSD) in stability testing of traditional Chinese medicine fingerprints are indicators for measuring the consistency of analytical methods. The purpose of RSD calculation is to evaluate the fluctuation of repeated experimental results under the same conditions. The calculation formula is: $RSD(\%) = (\frac{SD}{\overline{X}}) \times 100\%$,SD is the standard deviation of the sample, $\overline{X}$ is the average value of the sample.relative standard deviation (% RSD) were calculated using an Excel sheet.

Reference sources:Gohar, M.S.; Rahman, T.U.; Bahadur, A.; Ali, A.; Alharthi, S.; Al-Shaalan, N.H. Development and Validation of Novel HPLC Methods for Quantitative Determination of Vitamin D3 in Tablet Dosage Form. Pharmaceuticals 2024, 17, 505. https://doi.org/10.3390/ph17040505

5. In the abstract, the full name of the plant should be used for the first time, and O. opipara in the rest. be extended to the whole article and it should be written in italics like in line 102.

Response: Thank you for your constructive suggestion on the naming convention for "*Osbeckia opipara*" We have used the full name in the first mention within the abstract and subsequently referred to it as "*O. opipara*" throughout the article. We've also ensured it is italicized, consistent with the format in line 102.

6. The first paragraph of the introduction should be written more briefly.

Response: Thank you for your insightful suggestion. I have shortened the first paragraph of the introduction as requested and placed it in the text.

7. Check the tables, it seems that table 3 is missing.

Response: Thank you very much for your careful and kindly review. We have reviewed the manuscript again and added Table 3.

8. Abbreviations should be written in parentheses for the first time, such as RSD in lines 161 and 168.

Response: Thank you for pointing out the use of abbreviations in our manuscript. We have made modifications to the text and added a list of abbreviations.

9. To improve our understanding of the chromatogram, please demonstrate the 13 common peaks in Figure 8.

Response: Thank you very much for your constructive suggestions. We have marked the positions of 13 common peaks as required and have included them in the manuscript.

10. The text has some spelling and grammatical errors. Therefore, it is suggested to be edited by a native English writer.

Response: Thank you very much for your insightful suggestion.The manuscript has been edited by a native English editor.

2 Revision 1

2 comments · 25 Jun 2024 – 25 Jun 2024

Hadi Ghanbari Reviewer

ACCEPT 25 Jun 2024

The authors of this article (Manuscript ID: 146396) have addressed the comments as requested, and in my opinion, the article is of sufficient quality to be accepted.

Associate Editor

ACCEPT 25 Jun 2024

Accept