Peer Review File

Reviewer A

Please find in the following my comments about the review of a manuscript under the title (Investigating the multitarget pharmacological mechanism of Rhodiola wallichiana var. cholaensis acting on angina pectoris using combined network pharmacology and molecular docking). In this study, the authors aim to explore RW in the treatment of AP and to identify the potential mechanism of the core compounds.

Originality and relevance
♣ The study has moderate scientific quality.
♣ The study is relevant to the scope of this journal.
♣ The manuscript is relevant to the field and its presentation needs minor modifications to be clearer.

Comment 1:
Abstract:
♣ Add more details about the methodology and mention the most prominent finding in the results.
Reply 1: Thank you for your suggestions, we have modified our text as advised (see Page 2, line 46-59)"
Changes in the text:
Methods: In this study, systematic and comprehensive network pharmacology and molecular docking were used for the first time to explore the potential pharmacological mechanisms of RW on AP. First, the relative compounds were obtained by mining the literature, and potential targets of these compounds using target prediction were collected. We then built the AP target database using the DigSee and GeneCards databases. Based on the data, overlapping targets and hub genes were identified with Maximal Clique Centrality (MCC) algorithm in Cytoscape, cytoHubba. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses and protein-protein interaction (PPI) analysis were performed to screen the hub targets by topology. Molecular docking was utilized to investigate the receptor-ligand interactions on Autodock Vina and visualized in PyMOL
Results: A total of 218 known RW therapeutic targets were selected. Systematic analysis identified 9 hub targets (*VEGFA*, *GAPDH*, *TP53*, *AKT1*, *CASP3*, *STAT3*, *TNF*, *MAPK1* and *JUN*) mainly involved in the complex treatment effects associated with the protection of the vascular endothelium, as well as the regulation of glucose metabolism, cellular processes, inflammatory responses, and cellular signal transduction. Molecular docking indicated that the core compounds had good affinity with the core targets.

Comment 2:
Introduction
♣ The rationale of the study is not clear in the introduction section.
Reply 2: Thank you for your suggestions, we added some descriptions (see Page 3, line 96-97)"
Changes in the text:
Network pharmacology is a new research method that integrates pharmacodynamics, pharmacokinetics, and network analysis. In recent years, it has been applied to elucidate the possible mechanism of TCM prescriptions in the treatment of various diseases from the perspective of proteomics systems (5-7). In particular, it has been used to characterize the

interaction relationship of TCM in multi-components and multitargets, as well as to study the mechanism of multitarget compounds affecting the biological network of TCM (8,9). In this study, we explore the hub active ingredients and potential mechanisms of RW in AP based on network pharmacology and molecular docking.

Comment 3:
Materials and methods
♣ Add references for all methods used.
Reply 3: Thank you for your suggestions, we added references for all methods used.

Comment 4:
Results:
♣ The title and legends of the figures and tables should be informative and self-explanatory? Revise.
♣ Adjust the place of Figure 2 and Figure 3 legends.
Reply 4: Thank you for your suggestions, we have modified our text as advised.

Comment 5:
Discussion:
♣ The discussion needs more interpretation of the results.
Reply 5: Thank you for your suggestions, we have re-checked the discussion section, which includes all the results and analysis of the study, which can indicate the purpose of this study. Perhaps our analysis is not comprehensive enough, please teacher guidance.

Reviewer B

The paper titled "Investigating the multitarget pharmacological mechanism of Rhodiola wallichiana var. cholaensis acting on angina pectoris using combined network pharmacology and molecular docking" is interesting. The results of this study preliminarily identify the potential targets and signaling pathways of RW in AP therapy and lay a promising foundation for further experimental studies and clinical trials. However, there are several minor issues that if addressed would significantly improve the manuscript.

Comment 1: Suggest increasing the efficacy and safety evaluation of RW for treating angina pectoris, which may make the entire study more complete.
Reply 1: Thank you for your suggestions. RW has been in clinical use for many years and is described in the introduction. According to your suggestion, we searched relevant literature and found that the efficacy and safety of Teng S and Qian X (DOI:10.1097/md.0000000000031736)have been evaluated based on the data of clinical use, and the results were obtained based on clinical data analysis, which can better reflect the efficacy and safety evaluation results compared with experimental animal data.

Comment 2: Some fonts need to be enlarged, as shown in Figures 2,3,6.
Reply 2: Thank you for your suggestions. We have modified it according to your suggestion.

Comment 3: Suggest increasing the analysis of differential metabolites closely related to AP pathological processes and RW effects.

Reply 3: Thank you for your suggestions. Since this paper is only based on network pharmacology and molecular docking analysis of the mechanism of RW in the treatment of AP, neither grouping analysis nor metabolomics analysis has been conducted, so the analysis of differential metabolites cannot be carried out. But thank you for your suggestions, which provide research ideas for our subsequent research.

Comment 4: Figures 1-4 are not clear enough. It is recommended to provide clearer figures again.
Reply 4: Thank you for your suggestions. Due to the distortion of the pictures inserted into the manuscript, we have submitted the clear pictures as separate files.

Comment 5: Suggest increasing in vitro and in vivo experiments to prove the research results, which may be more credible.
Reply 5: Thank you for your suggestions. We also hope to verify it through experiments, but due to the limitation of experimental conditions, we cannot carry out relevant in vivo and in vitro experiments for verification in the short term. However, according to your suggestion, we will conduct further research in combination with clinical practice.

Comment 6: It is suggested to increase the analysis and functional research of key genes, which may be more convincing and meaningful.
Reply 6: Thank you for your suggestions. In the discussion in the original article, we performed functional analysis on the 9 core genes analyzed. Due to the limitations of experimental conditions, functional experimental studies cannot be carried out. We will conduct further functional and verification experimental studies on it based on your suggestions.

Comment 7: The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as "Herba Rhodiolae alleviates depression via the BDNF/TrkB-GSK-3β signaling pathway, PMID: 35071452". It is recommended to quote the articles.
Reply 7: Thank you for your suggestions to improve my article. Based on your suggestions, we have quoted the article in the introduction.

Comment 8: The latest application progress of network pharmacology and molecular docking in Chinese medicine research should be added to the discussion.
Reply 8: Thank you for your suggestions. We have added to the discussion the progress in the application of network pharmacology and molecular docking in traditional Chinese medicine research. (See Pag11-12 line367-375)
Changes in the text: At present, network pharmacology is mostly used in the screening of drug active ingredients, prediction of the mechanism of action of specific drugs, analysis of targets of main active ingredients, and development of combination drugs. As a research idea, network pharmacology can also be used to explain the compatibility rules of traditional Chinese medicine compounds and discover new indications of traditional Chinese medicine. Network pharmacology is becoming a powerful and attractive tool to reflect the multi-component and multi-target characteristics of traditional Chinese medicine. Based on network pharmacology

methods, it can help explain many difficult problems in the material basis of traditional Chinese medicine efficacy.
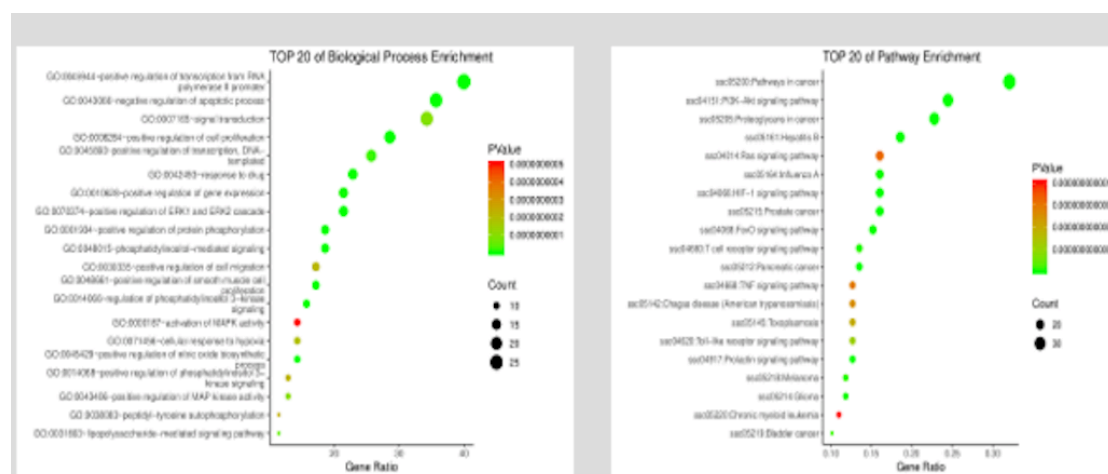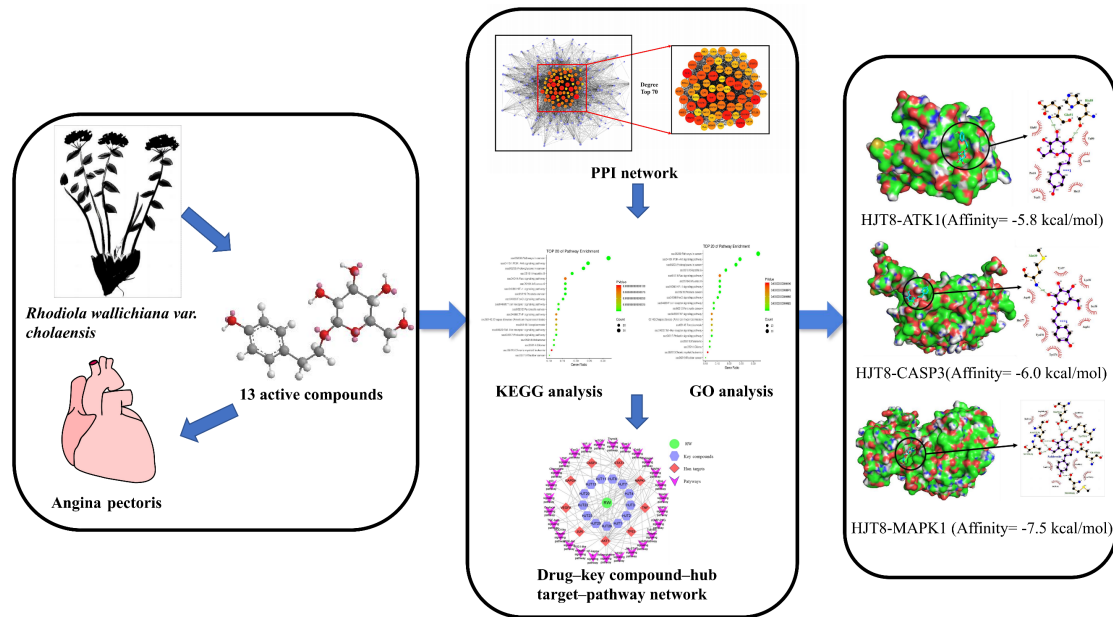
1. Figure 1

a. Please confirm whether these images were created by the authors. If they were downloading from the internet. To avoid any potential copyright issues, please confirm whether permission is needed. Please submit written permissions from the publisher to reuse them; otherwise, please delete them.
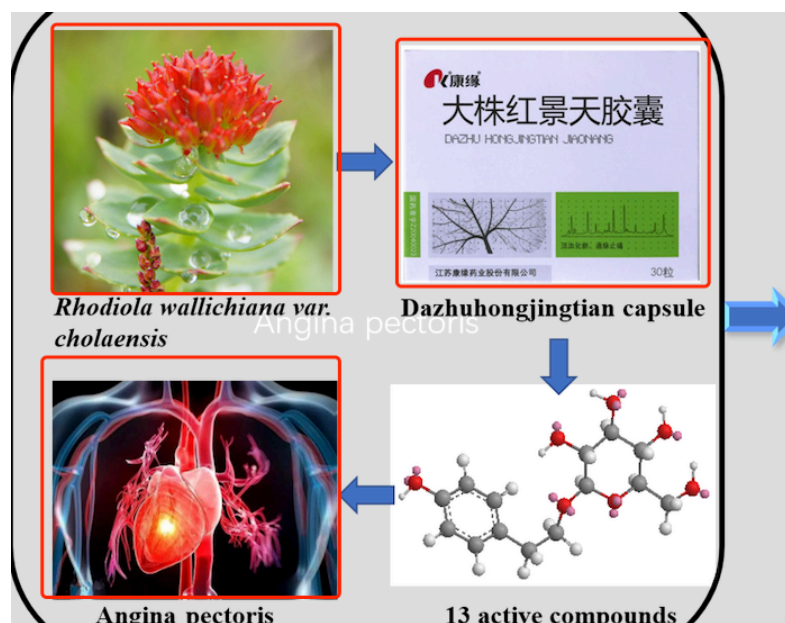
b. Please re-send us Figure 1 with higher resolution in JPG/TIFF, as the current one is not clear enough. Such as:
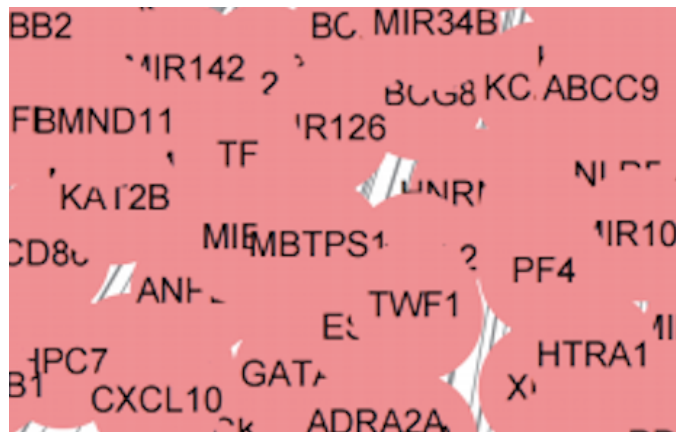

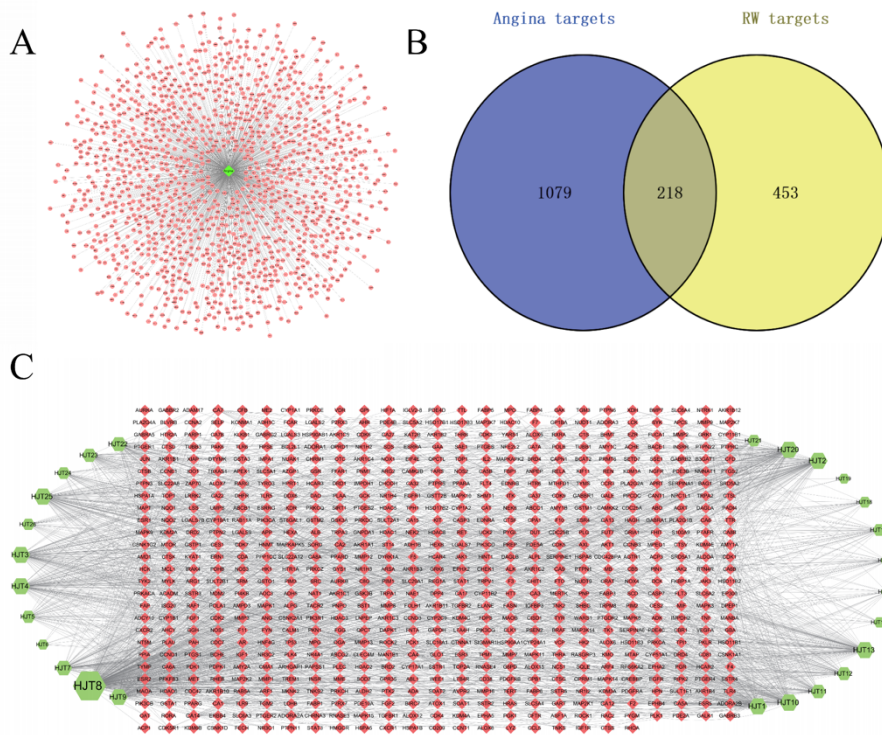Reply : Thank you for your review, it has been revised. Figure 1-revised

2. Figure 2A

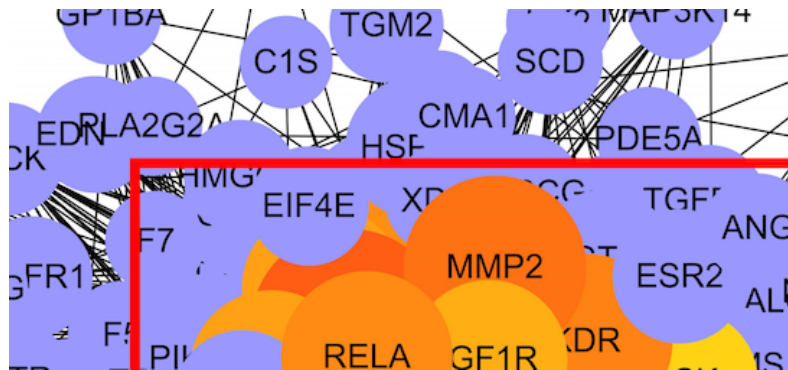The genes are overlapped. Please revise.

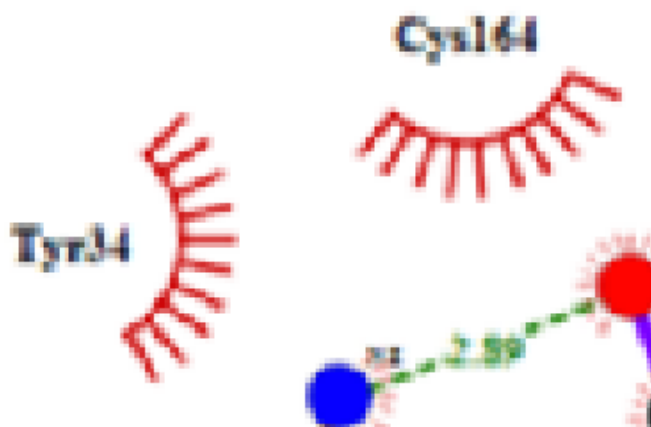Reply : Thank you for your review, it has been revised. Figure 2-revised

3. Figure 3A, B

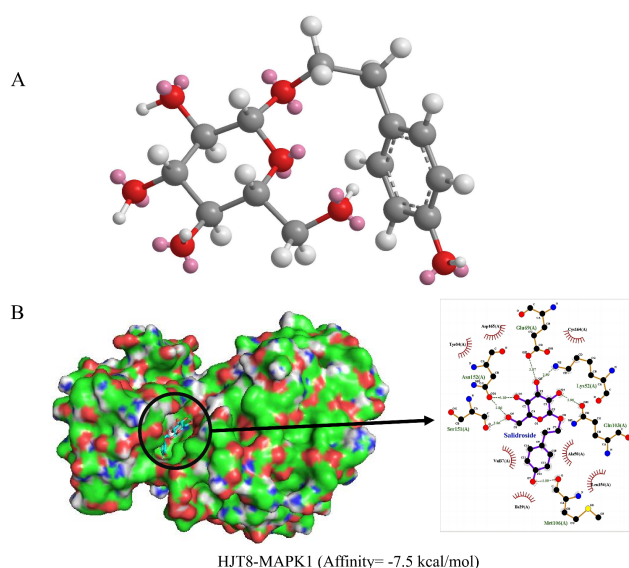The genes are overlapped. Please revise.

Reply : Thank you for your review, it has been revised. Figure 3-revised

4. Please re-send us **Figure 6B** with higher resolution in JPG/TIFF, as the current one is not clear enough.

Reply : Thank you for your review, it has been revised. Figure 6-revised

A

B

HJT8-MAPK1 (Affinity= -7.5 kcal/mol)

5. References

a. References 26 and 43 are the same. Please delete one of them and update the citations in the paper.

26. *Zhang GX, Zhang YY, Zhang XX, et al. Different network pharmacology mechanisms of Danshen-based Fangjis in the treatment of stable angina. Acta Pharmacol Sin 2018;39:952-60.*

43. *Zhang GX, Zhang YY, Zhang XX, et al. Different network pharmacology mechanisms of Danshen-based Fangjis in the treatment of stable angina. Acta Pharmacol Sin 2018;39:952-60.*

b. The authors mentioned "studies...", while only one reference was cited. <u>Change "Studies" to "A study" or add more citations.</u> Please revise. Please number references consecutively in the order in which they are first mentioned in the text.

- *Recent studies have demonstrated that the inflammatory response is related to the*

occurrence of CVDs such as coronary heart disease, which may cause local endothelial activation, atherosclerotic plaque rupture, and then thrombosis formation or rupture, leading to AP and myocardial infarction (55).

- *Multiple studies have shown that proinflammatory cytokines are significantly increased in patients with AP compared to healthy individuals. Meanwhile, it is important to note that this increased inflammatory activity may be related to the pathogenesis of AP. 1-Deoxynojirimycin (DNJ) significantly improves angina attack frequency by reducing the levels of inflammatory cytokines, including TNF-α(57).*

Reply :   Thank you for your review, it has been revised.