Research on antithrombotic mechanism of Paeoniae Radix Alba based on data mining, network pharmacology and molecular docking technology

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Abstract. The antithrombotic mechanism of Paeoniae Radix Alba (PRA) was studied with data mining, network pharmacology and molecular docking techniques. The active ingredient of PRA was through mining and screening TCMS database and literature, using the SwissTargetPrediction website to predict the target of the material. The Genacard database was used to mine "thrombotic" related targets, and the Cytoscape3.7.2 was used to construct PRA "active ingredients-thrombotic targets" network relationships. The target of antithrombotic drug was obtained by R language, Wencketty Diagram was drawn, and Kegg enrichment analysis was carried out. Then the core target of antithrombotic drug was selected by insertion of Cytonca, and its binding property to the core target was verified by molecular docking method. A total of 14 active components were selected from PRA and 257 targets were selected, among which 83 were antithrombotic targets and a total of 13 core antithrombotic targets were obtained, namely AKT1, ESR1, SRC, SERPINE1, MMP2, JUN, PTGS2, EGFR, FGF2, KDR, GAPDH, MMP9, VEGFA. A total of 1830 GO enrichment results were obtained, of which 1711 were enriched in biological processes (BP), 99 in molecular functions (MF) and 17 in cellular components (CC). A total of 127 KEGG signaling pathways were enriched. The docking results further showed that the active components of PRA could form intermolecular hydrogen bonds with the Amino acid residues of the core targets, and had strong binding properties. Based on data mining, network pharmacology and molecular docking techniques, PRA has been proved to have the characteristics of antithrombotic multi-pathway and synergistic therapy, and the possible targets of PRA antithrombotic and its mechanism of action were explored. It provides a theoretical basis for its further experimental study and clinical application.
1. Introduction
Paeoniae Radix Alba (PRA) is the dried root of the ranunculaceae plant Paeonia lactiflora. It has the effects of nourishing blood and restraining yin, softening the liver and relieving pain, and suppressing liver yang. It was commonly used to treat headache, dizziness, hypochondriac pain, abdominal pain, limb contracture, blood loss of green, irregular menstruation, night sweats, night sweats and so on. PRA is rich in chemical ingredients, including saponins, flavonoids, tannins, volatile oils, polysaccharides, etc. It has anti-inflammatory, antioxidant, antispasmodic, and liver protection and other pharmacological effects[1]. PRA has a long history of medicinal use in China, and hundreds of ancient prescriptions use PRA as the main medicine. PRA has many chemical components, and its pharmacological effects and clinical applications are also extensive. Studies have shown that the active ingredients of PRA have a pronounced antithrombotic impact. At present, the mechanism of PRA’s antithrombotic effect is not clear. On this basis, this paper analysed Paeonia Lactiflora Pall. By network pharmacology method and molecular docking technology Method.

1.1. Active ingredients of PRA
PRA components were retrieved from TCMSP database, and active components were screened according to oral bioavailability OB ≥30% and drug similarity DL ≥0.18. Literature review was conducted to supplement other active components. Targets of PRA active ingredients
The 2D structures of PRA active molecules were downloaded from PubChem website and then imported into SwissTargetPrediction website to predict and screen the targets of action of PRA active ingredients.

1.2. Antithrombotic targets of PRA
Searching genecard database with "Thrombus" as the key word and using correlation score ≥1 as the screening criteria, thrombus targets were obtained. The intersection of thrombosis targets and PRA active ingredient targets was taken to construct Venn diagrams to get the antithrombotic targets of PRA active ingredients.

1.3. Construction of PRA active ingredient-antithrombotic target network relationship diagram
Construction of Network Diagram of hyperactive components-antithrombotic targets in Cathoscape 3.7.2.

1.4. Construct a protein-protein interaction (PPI) network diagram and screen core genes
The antithrombotic target of PRA was imported into the string web site, the minimum confidence level was set to 0.9, idle nodes were deleted, PPI data tables were downloaded and imported into the CYTOCA 3.7.2 software, the node attributes are analyzed by using the Cytonca plug-in to screen the core genes.

1.5. GO and KEGG enrichment analysis
Go enrichment analysis and KEGG enrichment analysis of the antithrombotic targets of the APIs were performed by using the Biomanager software package of R language.

1.6. Docking of the active ingredients of PRA with the core antithrombotic target
The core target acts as the receptor protein and the PRA raw material acts as the ligand molecule. The receptor proteins were downloaded from the PDB database and the ligand molecules were downloaded from PubChem. The docking of Ligand and receptor protein was carried out by Sybyl X2.0 software. The receptor proteins were processed for hydrogen atom addition and water molecule removal. The active pockets are generated by automated modeling, and the ligand molecules and receptor protein active pockets are docked by the Surflex Dock (SFXC) method.

2. Results and Analysis
2.1. Active ingredients of PRA
A total of 14 PRA active ingredients were obtained (Table 1).
Table 1. Potential active ingredients of PRA

| Mol ID   | Molecule Name                                               | MW     | OB (%) | DL  |
|----------|-------------------------------------------------------------|--------|--------|-----|
| MOL001910 | 11alpha,12alpha-epoxy-3beta-23-dihydroxy-30-norolean-20-en-28,12beta-olide | 470.71 | 64.77  | 0.38|
| MOL001918 | paeoniflorgenone                                            | 318.35 | 87.59  | 0.37|
| MOL001919 | palbinone                                                   | 358.52 | 43.56  | 0.53|
| MOL001921 | lactiflorin                                                | 462.49 | 49.12  | 0.8 |
| MOL001924 | paeoniflorin                                               | 480.51 | 53.87  | 0.79|
| MOL001925 | paeoniflorin_qt                                            | 318.35 | 68.18  | 0.4 |
| MOL001928 | albiflorin_qt                                              | 318.35 | 66.64  | 0.33|
| MOL001930 | benzoyl paeoniflorin                                       | 584.62 | 31.27  | 0.75|
| MOL000211 | mairin                                                     | 456.78 | 55.38  | 0.78|
| MOL000358 | beta-sitosterol                                             | 414.79 | 36.91  | 0.75|
| MOL000359 | sitosterol                                                  | 414.79 | 36.91  | 0.75|
| MOL000422 | kaempferol                                                 | 286.25 | 41.88  | 0.24|
| MOL000492 | cianidanol                                                  | 290.29 | 54.83  | 0.24|
| MOL001927 | albiflorin                                                 | 480.46 | 12.09  | 0.77|

2.2. Targets of PRA active ingredients
A total of 257 prediction targets of PRA active ingredients were obtained.

2.3. Antithrombotic targets of PRA
A total of 1132 thrombus targets were obtained, and Venn diagrams (Fig. 1) were cross-plotted with the superapi targets to obtain 83 super antithrombus targets.

2.4. active ingredients-antithrombotic targets network relationship diagram
A network diagram of PRA active ingredients-antithrombotic targets was constructed by Cytoscape 3.7.2 software. The blue circles on the left were the active components of PRA, and the green squares on the right were the disease genes. The graph had 93 nodes and 146 edges (Figure 2), reflecting the complex interactions of PRA with multiple components and multiple targets.

2.5. protein-protein interaction (PPI) network diagram and core genes
As shown in figure 3, the PPI network with 83 nodes and 710 edges has hyperactive antithrombotic targets. There are complex interactions among the 83 antithrombotic targets, and most of them have one or more interactions. The above-mentioned target files are imported into the software of cell landscape 3.7.2, the characteristics of the nodes are analyzed by using the plug-in Cytoscape, and the parameters of each node are larger than the median as the filtering condition, they were AKT1, esr1, SRC, serpine1, mmp2, Jun, ptgs2, egfr, F2, KDR, Gapdh, MMP9, GFFA and so on.
Figure 1. Venn diagram of PRA active ingredient targets and thrombotic targets

Figure 2. PRA active ingredients-antithrombotic targets network relationship diagram
2.6. **GO enrichment analysis and KEGG enrichment analysis**

PRA antithrombotic targets were annotated by GO function as biological process (GO-BP), molecular function (GO-MF) and cellular composition (GO-CC), respectively. A total of 1,830 entries were enriched and annotated. There are 1,711 items in go-bp, among which 1,514 items are adjusted by $P \leq 0.05$, 99 items are enriched by GO-MF, among which 87 items are adjusted by $P \leq 0.05$, 20 items are enriched by GO-CC which 17 items are adjusted by $P \leq 0.05$. Figure 4 shows the top 10; the deeper the red, the more pronounced the enrichment, the larger the circle, the more enriched genes.

![Diagram of protein-protein interaction network](image-url)
Kegg enrichment analysis showed that the antithrombotic targets of PRA were rich in 127 pathways. The number one pathway was the tyrosine-kinase inhibitor resistance, Proteoglycan in cancer, endocrine resistance, relaxin signaling pathway.
### 2.7. Docking of the active ingredients of *Radix Paeoniae Alba* with the core antithrombotic target

The molecular docking results showed that ligand molecules (PRA active ingredients) could form hydrogen-bonded interactions with amino acid residues of the core antitumor target. These results also further validate the reliability of the network pharmacology predictions (Table 2).

**Table 2.** The docking results of the active ingredients of PRA and the core target molecules

| Ingredients         | 4KZN Total score | Sites     | 4OEE Total score | Sites     | 3G5Y Total score | Sites     |
|---------------------|------------------|-----------|------------------|-----------|------------------|-----------|
| Lactiflorin         | 3.11             | ARG23     | 3.72             | GLU96\GLY6| GLU96\GLY6\GLY6| 5.23      |
| Paeoniflorin        | 2.17             | GLU30     | 3.87             | LEU98\AMN1| LEU98\AMN1\GLU98| –         |
| Paeoniflorin_qt     | 2.22             | GLU30\HIS27\ARG23 | 3.87 | LEU98\AMN1\GLU30 | –         |
| Albiflorin          | 3.39             | HIS27\AFG23\GLU30 | 2.79 | ARG97\AMN1\ARG60 | –         |

### 3. Discussion

Thrombosis is a condition in which blood vessels become partially or completely blocked due to excessive clotting. Due to the distribution of blood clots in different parts of the body, the morbidity and mortality of cerebrovascular, cardiovascular and peripheral vascular diseases are on the rise, so the key to treat thrombotic diseases is to prevent the formation of blood clots. It was found that Paeoniflorin and Paeoniflorin could inhibit the formation of thrombus, and their antithrombotic effects were related to the regulation of endothelial active substances, activation of blood flow and anticoagulation. The results of this study suggest that Paeoniflorin may regulate P38 and MAPK 8 signaling pathways, up regulate the expression of Plasminogen activator and improve prethrombotic state and recanalization.

Shear stress-induced platelet aggregation (SIPA) is a promising target for overcoming bleeding. Thien Ngo found that paeoniflorin has a significant inhibitory effect on shear-induced human platelet aggregation, paeoniflorin can regulate vascular blood disease, and the interaction of factor-platelet glycoprotein Ib (GP Ib) has a highly selective inhibitory effect on SIPA. Paeoniflorin can be used as a new type of antiplatelet drug to selectively target SIPA with higher safety. J Ye evaluated the antithrombotic effect of paeoniflorin in a photochemical reaction microvascular thrombosis model. The antithrombotic effect of Paeoniflorin may be related to the inhibition of Arachidonic acid metabolism, the increase of tissue type Plasminogen activator activity and the protection against free radicals. The results of these studies have further verified the pharmacological effects of PRA Antithrombotic.

In this study, PRA screened a total of 14 active ingredients and 257 disease targets, of which 83 were antithrombotic targets and 13 were core targets, namely AKT1, ESR1, SRC, SERPINE1, MMP2, JUN, PTGS2, EGFR, FGF2, KDR, GAPDH, MMP9, and VEGFA. AKT1 is a protein kinase encoding one of the three members of the human AKT serine-threonine protein kinase family (AKT1, AKT2 and AKT3), which regulates many processes, including cell metabolism, proliferation, growth and angiogenesis. A total of 1830 PRA antithrombotic targets were annotated by GO, of which 1711 were enriched in GO-BP, 99 in GO-MF, and 17 in GO-CC. KEGG pathway enrichment analysis showed that the antithrombotic effect of PRA was associated with resistance to EGFR tyrosine kinase inhibitors, proteoglycans in cancer, endocrine resistance, relaxin signaling pathway, lipid and atherosclerosis, HIF-1 signaling pathway, serotonomic synapse; the AGE-RAGE signaling pathway is strongly associated with other ways involved in diabetic complications. The results of molecular docking further confirmed that the active components could form intermolecular hydrogen bonds with the Amino acid residues of the core targets and have strong binding properties.
4. Conclusion
Traditional Chinese Medicine (TCM) is rich in chemical components for treating diseases, and its complex chemical structure and multiple action targets constitute its unique clinical efficacy. In this study, data mining, network pharmacology and molecular docking technology research methods were used to explore the effective components, targets and pathways of PRA antithrombotic to provide reference for clinical practice.

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