Study on the Anti-inflammatory Mechanism of volatile oil of Amydrium sinense based on network pharmacology

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Abstract. Amydrium sinense has been widely used to treat rheumatism are in ethnic areas. The modern studies have shown that rheumatism is closely related to inflammation, and volatile oil of traditional Chinese medicine has anti-inflammatory activity, but the mechanism is not fully elucidated. In this study, the potential mechanism of anti-inflammatory effect of Amydrium sinense was systematically explored by using the network pharmacology. Firstly, the active chemical ingredients of Amydrium sinense were prescreened according to ADME parameters (OB≥30% and DL≥0.18) and the Pharmacological activity. The potential targets were screened with the databases of TCMSP. Then the “component-target-disease” network was constructed using Cytoscape 3.6.1 software. Finally, GO (gene function) enrichment analysis were carried out by biological information annotation database (DAVID), and signal pathway analysis were executed by KEGG Pathway database. The network analysis revealed that 27 compounds were screened as active compounds; 193 targets were searched, of which 38 potential targets the most were closely related to the prevention and treatment of inflammation. In addition, it suggested that 212 biological processes and 79 signalling pathways were screened. Among them, the signalling pathway most closely related to the relevant regulated of NF-kB, TNF, Toll-like receptor signalling pathway, Hepatitis B and Inflammatory response, etc. Its anti-inflammatory mechanism may coordinate with each other through multiple processes to exert anti-inflammatory biological effects. The above results provide strong support for studying the molecular mechanism of Amydrium sinense in the treatment of inflammation.

1. Introduction
The dried rattan cane of Amydrium Haines (Engl.) H. Li. is Amydrium sinense, which is attached to the tree trunk or cliff of broad-leaved evergreen forests. It mainly distributed in the southwest, Hubei, Hunan, Guangxi and in southern Guizhou of China. Amydrium sinense has the effect of soothe the channels and quicken the network vessels, removing blood stasis to kill pain [1]. It is mainly used to treat the disease of fractures, traumatic injury, angina formula, wind-dampness and numbness, etc. The
main chemical components are alkaloids, flavonoids, lactone, coumarin, volatile oil, cardiac glycoside, steroid, etc [2-3]. The drug is also called "rheumatic medicine" and commonly used in ethnic areas of China. Modern studies have shown that rheumatism is closely related to anti-inflammatory [4-5], and the volatile oil of traditional Chinese medicine also has anti-bacterial, anti-inflammatory, anti-oxidative, anti-tumor, and other pharmacological activities [6-7]. However, the medicine is mainly the chemical constituents extract, spectral identification and microscopic identification [8-11], but the anti-inflammatory mechanism is unclear. Therefore, it is helpful to promote the further development of ethnic medicine for revealing the anti-inflammatory mechanism of Amydrium sinense.

In recent years, the rise of network pharmacology has brought new technologies and methods to the study of traditional Chinese medicine. It combines new technologies with multidisciplinary integration that include systems biology, polypharmacology, systems biology, network analysis, proteomics, genomics, metabolomics, etc. This coincides with holistic and systematic characteristics research concepts and the “multi-component, multi-target and multi-pathway” synergistic features of traditional Chinese medicine. Network pharmacology can construct a multi-level network, comprehensively explore the relationship between drugs and diseases, so that traditional Chinese medicine can regulate the body from the overall level to perform treating effect [12-13]. Therefore, in this article, the possible targets, signaling pathways and the mechanism of preventing and treating inflammation were excavation and demonstration in Amydrium sinense, which based on network pharmacology. It provides a reference for the further development and research of the anti-inflammatory mechanism of Amydrium sinense. The technical route is shown in Figure 1.

![Figure 1. The technical route.](image)

2. Method

2.1. Chemical composition database construction
The all volatile oil components were obtained from the relevant literature (CNKI, PubMed). Then, the action chemical constituents were screened by TCMSp database with ADME parameters (OB≥30% and DL≥0.15) and relevant pharmacological activities reported in literature as standard [14-19]. The action constituents corresponding to chemical structural formula was obtained from TCMSp database.
2.2. Molecule structure and target protein prediction
Through TCMSP database and a database of Chinese medicine target database to synthetically analysis by p-value as index. Then, the database of Chinese herbal medicine (HIT, http://lifecenter.sgst.cn/hit/) and the control target database (TTD, http://bidd.nus.edu.sg/group/cjttd/) were used to screen the active constituents. The gene and protein targets for the treatment of inflammation were screened by a comprehensive database of human genes and gene phenotypes (OMIM, http://www.omim.org/). The human target connexins were obtained from an interactive protein database (http://dip.doe-mbi.ucla.edu). Finally, all the selected targets were transformed into UniProt ID format by the UniProt database (https://www.uniprot.org/).

2.3. Network construction and topologic profile analysis
The active constituents, the targets corresponding to the Amydrium sinense, inflammatory targets and relevant protein targets were connected as the “component-target-disease” network. The above network was visually analyzed by using Cytoscape 3.6.1 software, then acquired the topological parameters of Degree, Betweenness and Closeness centrality. They were used to evaluate by CentiScaPe 1.2. In this study, targets with more than three topological parameter values were used as candidate targets.

2.4. Biological progress and pathway enrichment analysis
Interactions between these targets are constructed by STRING (http://string-db.org/). Then, the database DAVID (https://david.ncifcrf.gov/) was carried out to analysis the KEGG pathway and the biological process of GO (Gene Ontology).

3. Result

3.1. These active constituents screened
The total 72 chemical constituents of volatile oil obtained with literature review (CNKI database, bMed database) and TCMSP database. Afterwards, 27 active components were screened by a standard of ADME parameters (OB≥30% and DL≥0.18) and the pharmacodynamics activities, the results are shown in table 1.

| Mol ID    | Chemical constituents | target | construction |
|-----------|-----------------------|--------|--------------|
| MOL000667 | 1-hexanol             | 1      |              |
| MOL001335 | benzyl alcohol        | 11     |              |
| MOL004582     | 1-methylnaphthalene | 2 |
|---------------|---------------------|---|
| MOL000259     | carvacrol           | 10|
| MOL005577     | hendecanal          | 1 |
| MOL005483     | 2-methylnaphthalene | 1 |
| MOL003127     | germacrene D        | 4 |
| MOL001167     | β-selinene          | 11|
| MOL003028     | eudesmol            | 6 |
| MOL004358     | linalool            | 4 |
| MOL000116 | nonanal | 4 |
|-----------|---------|---|
| MOL003493 | naphthalene | 11 |
| MOL000261 | myristicin | 29 |
| MOL00269 | elemicin | 22 |
| MOL002504 | nerolidol | 2 |
| MOL000875 | αcedrol | 5 |
| MOL000879 | methyl palmitate | 9 |
| MOL011081 | linolenic acid methyl ester | 2 |
| MOL000700 | nerol     | 1 |
|-----------|-----------|---|
| MOL000703 | Heptan-2-one | 1 |
| MOL000668 | 2-n-pentylfuran | 2 |
| MOL001417 | Trans-2-octenal | 3 |
| MOL000716 | (E)-non-2-enal | 6 |
| MOL001226 | Alpha-terpineol | 3 |
| MOL000908 | Beta-elemene | 24 |
| MOL000936 | Germacrene B | 5 |
3.2. Network construction

The OMIM database was used as screened out 778 targets of inflammation-related. The anti-inflammatory interactive network was constructed by Cytoscape 3.6.1. A total of 404 interacting proteins were screened. After visualizing with different colors and shapes, the network relationship between active components and disease targets could be directly seen in figure 2.

![Network Diagram](image_url)

**Figure 2.** “Component-Target-Disease” Interactive Network for Anti-inflammatory Action of Amydrium sinense. (The yellow square and yellow ellipse have 122, while represented the target for drugs and diseases, also represented the significant target for Amydrium sinense to anti-inflammatory; the red triangle has 59 that represented the predicted active chemical components of Amydrium sinense; the blue ellipse have 51 that represented the direct target of active constituents of Amydrium sinense; and the purple ellipse has 678 that represented the interacting protein connected the components of Amydrium sinense with the disease targets.)

3.3. Topological profile analysis

Combined with Cytoscape 3.6.1 interactive network analysis, the protein targets associated with the active components of the drug were screened, and the topological parameters were calculated for these protein targets. Taking all of the median value (Degree, Betweenness centrality and Closeness centrality) in the network as calculation results, while got the three topological parameters are: 4, 0.007 and 0.181. Based on all the values higher than the median value of nodes were the important target proteins, 38 targets were screened by the topological parameters, the results were shown in Table 2. Through the analysis of STRING database, the relationship between the target proteins is shown in Figure 3. The potential targets play an important role in the anti-inflammatory process in the figure, such as Cellular tumor antigen p53, NF-kappa-B essential modulator, TNF receptor-associated factor 6, Transcription factor p65, Nuclear factor NF-kappa-B p105 subunit, Epidermal growth factor receptor, NF-kappa-B inhibitor alpha.
Table 2. Topological parameters related to the Target of Anti-inflammatory effects of active components of Amydrium sinense.

| Uniprot ID | Protein names | Gene names | BetweennessCentrality | Closeness Centrality | Degree |
|------------|---------------|------------|------------------------|----------------------|--------|
| P04637    | Cellular tumor antigen p53 | TP53 | 0.37817466 | 0.29023384 | 61 |
| Q9Y6K9    | NF-kappa-B essential modulator | IKBKG | 0.05461217 | 0.24421296 | 23 |
| Q9Y4K3    | TNF receptor-associated factor 6 | TRAF6 | 0.06017649 | 0.2296807 | 22 |
| Q04206    | Transcription factor p65 | RELA | 0.06260179 | 0.23401109 | 20 |
| P19838    | Nuclear factor NF-kappa-B p105 subunit | NFKB1 | 0.02141255 | 0.21385135 | 20 |
| P00533    | Epidermal growth factor receptor | EGFR | 0.05797635 | 0.23169839 | 19 |
| P25963    | NF-kappa-B inhibitor alpha | NFKBIA | 0.04080834 | 0.23734533 | 18 |
| O15111    | Inhibitor of nuclear factor kappa-B kinase subunit alpha | CHUK | 0.02395997 | 0.23418424 | 18 |
| O14920    | Inhibitor of nuclear factor kappa-B kinase subunit beta | IKBKB | 0.02354588 | 0.24525378 | 15 |
| P05412    | Transcription factor AP-1 | JUN | 0.07648752 | 0.22351695 | 14 |
| P31749    | RAC-alpha serine/threonine-protein kinase | AKT1 | 0.03796852 | 0.23453131 | 13 |
| Q99558    | Mitogen-activated protein kinase kinase 14 | MAP3K14 | 0.02240681 | 0.22860238 | 13 |
| P35354    | Prostaglandin G/H synthase 2 | PTGS2 | 0.06579843 | 0.22574983 | 12 |
| Q16665    | Hypoxia-inducible factor 1-alpha | HIF1A | 0.03191507 | 0.18678076 | 12 |
| P25445    | Tumor necrosis factor receptor superfamily member 6 | FAS | 0.1058043 | 0.21678082 | 11 |
| P09874    | Poly [ADP-ribose] polymerase 1 | PARP1 | 0.04060065 | 0.22976407 | 11 |
| P24385    | G1/S-specific cyclin-D1 | CCND1 | 0.04395384 | 0.24123476 | 10 |
| Q99759    | Mitogen-activated protein kinase kinase 3 | MAP3K3 | 0.03079546 | 0.22009736 | 10 |
| P19438    | Tumor necrosis factor receptor superfamily member 1A | TNFRSF1A | 0.02181191 | 0.20108005 | 10 |
| P04150    | Glucocorticoid receptor | NR3C1 | 0.03466849 | 0.21530612 | 8 |
| P01375    | Tumor necrosis factor | TNF | 0.03383376 | 0.19551544 | 8 |
| P41182    | B-cell lymphoma 6 protein | BCL6 | 0.03137642 | 0.2249467 | 8 |
| Q86WV6    | Stimulator of interferon genes protein | TME1M73 | 0.00988679 | 0.22017391 | 8 |
| Q13546    | Receptor-interacting serine/threonine-protein kinase 1 | RIPK1 | 0.07265818 | 0.22591006 | 7 |
| P23219    | Prostaglandin G/H synthase 1 | PTGS1 | 0.02723218 | 0.2169294 | 7 |
| P37231    | Peroxisome proliferator-activated receptor gamma | PPARG | 0.0120563 | 0.1810123 | 7 |
| Q13158    | FAS-associated death domain protein | FADD | 0.01029643 | 0.18959227 | 7 |
| P05231    | Interleukin-6 | IL6 | 0.0157328 | 0.18476357 | 6 |
| Q14790    | Caspase-8 | CASP8 | 0.01123555 | 0.19597523 | 6 |
| P41279    | Mitogen-activated protein kinase kinase 8 | MAP3K8 | 1.63E-05 | 0.19251825 | 6 |
| Q13651    | Interleukin-10 receptor subunit alpha | IL10RA | 0.02818105 | 0.18519602 | 5 |
| O00482    | Nuclear receptor subfamily 5 group A member 2 | NR5A2 | 0.01426064 | 0.1993073 | 5 |
| P06396    | Gelsolin | GSN | 0.01260823 | 0.20210728 | 5 |
| Q03164    | Histone-lysine N-methyltransferase 2A | KMT2A | 0.0103907 | 0.20571986 | 5 |
| Q13191    | E3 ubiquitin-protein ligase CBL-B | CBLB | 0.0094368 | 0.22048067 | 5 |
| Q9C000    | NACHT, LRR and PYD domains-containing protein 1 | NLRP1 | 0.0464135 | 0.19664492 | 4 |
| P10145    | Interleukin-8 | CXCL8 | 0.025024 | 0.1809605 | 4 |
| Q16236    | Nuclear factor erythroid 2-related factor 2 | NFE2L2 | 0.01418379 | 0.2246274 | 4 |
3.4. **Biological function analysis of GO**

![Figure 4. GO of biological function Enrichment Analysis of Anti-inflammatory effect of Amydrium sinense distribution.](image-url)

**Figure 3.** Protein interaction Diagram of Anti-inflammatory effects of active components of Amydrium sinense.
Mapping 38 potential targets into the David database to enrich biological functions, and systematically analyze their biological processes. 212 biological processes were enriched, of which 33 biologic processes by threshold (P ≤ 0.00001) were shown in Table. Fig. 4. The results were showed that the main biological processes refer to targets include positive regulation of transcription from RNA polymerase II promoter, inflammatory response, positive regulation of I-kappaB kinase/NF-kappaB signaling, I-kappaB kinase/NF-kappaB signaling, cellular response to mechanical stimulus, etc.

**Table 3. GO of biological function Enrichment Analysis of Anti-inflammatory effect of Amydrium sinense.**

| Category | Term                                                                 | Count | Count% | P-Value  |
|----------|----------------------------------------------------------------------|-------|--------|----------|
| GOTERM_BP_DIRECT | positive regulation of transcription from RNA polymerase II promoter | 24    | 63.2   | 9.00E-20 |
| GOTERM_BP_DIRECT | inflammatory response                                                 | 16    | 42.1   | 8.80E-16 |
| GOTERM_BP_DIRECT | positive regulation of I-kappaB kinase/NF-kappaB signaling           | 12    | 31.6   | 3.10E-14 |
| GOTERM_BP_DIRECT | I-kappaB kinase/NF-kappaB signaling                                  | 9     | 23.7   | 5.80E-13 |
| GOTERM_BP_DIRECT | cellular response to mechanical stimulus                              | 9     | 23.7   | 2.40E-12 |
| GOTERM_BP_DIRECT | positive regulation of NF-kappaB transcription factor activity       | 10    | 26.3   | 9.60E-12 |
| GOTERM_BP_DIRECT | regulation of tumor necrosis factor-mediated signaling pathway       | 7     | 18.4   | 4.30E-11 |
| GOTERM_BP_DIRECT | death-inducing signaling complex assembly                            | 5     | 13.2   | 1.40E-09 |
| GOTERM_BP_DIRECT | apoptotic process                                                    | 13    | 34.2   | 1.70E-09 |
| GOTERM_BP_DIRECT | positive regulation of smooth muscle cell proliferation              | 7     | 18.4   | 3.40E-09 |
| GOTERM_BP_DIRECT | TRIF-dependent toll-like receptor signaling pathway                   | 6     | 15.8   | 3.70E-09 |
| GOTERM_BP_DIRECT | positive regulation of apoptotic process                             | 10    | 26.3   | 0.00000013 |
| GOTERM_BP_DIRECT | activation of cysteine-type endopeptidase activity involved in apoptotic process | 7 | 18.4 | 0.00000025 |
| GOTERM_BP_DIRECT | stimulatory C-type lectin receptor signaling pathway                 | 7     | 18.4   | 0.00000011 |
| GOTERM_BP_DIRECT | Fc-epsilon receptor signaling pathway                                | 8     | 21.1   | 0.00000011 |
| GOTERM_BP_DIRECT | positive regulation of transcription, DNA-templated                  | 11    | 28.9   | 0.00000011 |
| GOTERM_BP_DIRECT | stress-activated MAPK cascade                                         | 5     | 13.2   | 0.00000012 |
| GOTERM_BP_DIRECT | cellular response to tumor necrosis factor                           | 7     | 18.4   | 0.00000014 |
| GOTERM_BP_DIRECT | regulation of cell proliferation                                     | 8     | 21.1   | 0.00000014 |
| GOTERM_BP_DIRECT | necrototic signaling pathway                                          | 4     | 10.5   | 0.0000002 |
| GOTERM_BP_DIRECT | nucleotide-binding oligomerization domain containing signaling pathway | 5 | 13.2 | 0.00000024 |
| GOTERM_BP_DIRECT | negative regulation of apoptotic process                            | 10    | 26.3   | 0.00000046 |
| GOTERM_BP_DIRECT | T cell receptor signaling pathway                                     | 7     | 18.4   | 0.00000079 |
| GOTERM_BP_DIRECT | response to lipopolysaccharide                                       | 7     | 18.4   | 0.00000014 |
| GOTERM_BP_DIRECT | extrinsic apoptotic signaling pathway                                | 5     | 13.2   | 0.00000021 |
| GOTERM_BP_DIRECT | positive regulation of nitric oxide biosynthetic process             | 5     | 13.2   | 0.00000023 |
| GOTERM_BP_DIRECT | activation of cysteine-type endopeptidase activity involved in apoptotic signaling pathway | 4 | 10.5 | 0.00000028 |
| GOTERM_BP_DIRECT | cellular response to lipopolysaccharide                              | 6     | 15.8   | 0.00000046 |
| GOTERM_BP_DIRECT | response to muscle stretch                                            | 4     | 10.5   | 0.00000054 |
| GOTERM_BP_DIRECT | cellular response to DNA damage stimulus                             | 7     | 18.4   | 0.00000057 |
| GOTERM_BP_DIRECT | tumor necrosis factor-mediated signaling pathway                     | 6     | 15.8   | 0.00000057 |
| GOTERM_BP_DIRECT | regulation of extrinsic apoptotic signaling pathway via death domain receptors | 4 | 10.5 | 0.00000066 |
| GOTERM_BP_DIRECT | cellular response to organic cyclic compound                         | 5     | 13.2   | 0.00000083 |
3.5. Enrichment Analysis of signalling pathway

38 potential targets were mapped to the DAVID database for KEGG pathway analysis. 79 biological processes were enriched. Among these, 42 pathways were screened ($P \leq 0.00001$), the results were shown in Table 4 and Fig 5. These pathways are closely related to the mechanism of Amydrium sinense, such as TNF signaling pathway, Apoptosis, Chagas disease (American trypanosomiasis), Toll-like receptor signaling pathway, Hepatitis B, etc.

In the KEGG signaling pathway database, using KEGG mapper function to label 38 target protein on the signal pathway. The red marks respresented the anti-inflammatory targets, the green marks respresented pathway targets, the results are shown in figure 6. In the figure, we can found that there are several pathways lead to the active of NF-kappa B. The canonical pathway is induced by tumour necrosis factor-alpha (TNF-alpha), interleukin-1 (IL-1) or byproducts of bacterial and viral infections, the non-canonical pathway is triggered by particular members of the TNFR superfamily.

Figure 5. KEGG Pathway enrichment Analysis of Anti-inflammatory effect of Amydrium sinense distribution.
Table 4. KEGG Pathway enrichment Analysis of Anti-inflammatory effect of Amydrium sinense.

| Category                     | Term                                      | Count | Count% | P-Value       |
|------------------------------|-------------------------------------------|-------|--------|---------------|
| KEGG_PATHWAY                 | TNF signaling pathway                     | 18    | 47.4   | 5.9E-22       |
| KEGG_PATHWAY                 | Apoptosis                                 | 15    | 39.5   | 2.5E-20       |
| KEGG_PATHWAY                 | Chagas disease (American trypanosomiasis) | 16    | 42.1   | 1.2E-18       |
| KEGG_PATHWAY                 | Toll-like receptor signaling pathway      | 16    | 42.1   | 1.6E-18       |
| KEGG_PATHWAY                 | Hepatitis B                               | 16    | 42.1   | 2.1E-16       |
| KEGG_PATHWAY                 | Pathways in cancer                        | 21    | 55.3   | 5.3E-16       |
| KEGG_PATHWAY                 | RIG-I-like receptor signaling pathway     | 13    | 34.2   | 6.9E-16       |
| KEGG_PATHWAY                 | NF-kappa B signaling pathway              | 13    | 34.2   | 1.1E-14       |
| KEGG_PATHWAY                 | Hepatitis C                               | 14    | 36.8   | 7E-14         |
| KEGG_PATHWAY                 | Herpes simplex infection                  | 15    | 39.5   | 1.9E-13       |
| KEGG_PATHWAY                 | MAPK signaling pathway                    | 16    | 42.1   | 9.2E-13       |
| KEGG_PATHWAY                 | Osteoclast differentiation                | 13    | 34.2   | 1.7E-12       |
| KEGG_PATHWAY                 | Toxoplasmosis                             | 12    | 28.9   | 6.4E-12       |
| KEGG_PATHWAY                 | Small cell lung cancer                    | 11    | 28.9   | 1.3E-11       |
| KEGG_PATHWAY                 | HTLV-I infection                          | 15    | 39.5   | 1.8E-11       |
| KEGG_PATHWAY                 | Epstein-Barr virus infection              | 12    | 31.6   | 2E-11         |
| KEGG_PATHWAY                 | Epithelial cell signaling in Helicobacter pylori infection | 10    | 26.3   | 4.5E-11       |
| KEGG_PATHWAY                 | T cell receptor signaling pathway         | 11    | 28.9   | 6.7E-11       |
| KEGG_PATHWAY                 | Chronic myeloid leukemia                  | 10    | 26.3   | 8.9E-11       |
| KEGG_PATHWAY                 | Prostate cancer                           | 10    | 26.3   | 5.6E-10       |
| KEGG_PATHWAY                 | Cytosolic DNA-sensing pathway             | 9     | 23.7   | 1.1E-09       |
| KEGG_PATHWAY                 | Pancreatic cancer                         | 9     | 23.7   | 1.3E-09       |
| KEGG_PATHWAY                 | Adipocytokine signaling pathway           | 9     | 23.7   | 2.3E-09       |
| KEGG_PATHWAY                 | Non-alcoholic fatty liver disease (NAFLD) | 11    | 28.9   | 0.0000000004  |
| KEGG_PATHWAY                 | Influenza A                               | 11    | 28.9   | 0.000000016   |
| KEGG_PATHWAY                 | Measles                                   | 10    | 26.3   | 0.00000023    |
| KEGG_PATHWAY                 | B cell receptor signaling pathway         | 8     | 21.1   | 0.00000061    |
| KEGG_PATHWAY                 | Neurotrophin signaling pathway            | 9     | 23.7   | 0.0000017     |
| KEGG_PATHWAY                 | Tuberculosis                              | 10    | 26.3   | 0.0000027     |
| KEGG_PATHWAY                 | Legionellosis                             | 7     | 18.4   | 0.0000034     |
| KEGG_PATHWAY                 | Acute myeloid leukemia                    | 7     | 18.4   | 0.0000042     |
| KEGG_PATHWAY                 | Shigellosis                               | 7     | 18.4   | 0.0000095     |
| KEGG_PATHWAY                 | Insulin resistance                        | 8     | 21.1   | 0.0000014     |
| KEGG_PATHWAY                 | Leishmaniasis                             | 7     | 18.4   | 0.0000018     |
| KEGG_PATHWAY                 | Pertussis                                 | 7     | 18.4   | 0.0000024     |
| KEGG_PATHWAY                 | Viral carcinogenesis                      | 9     | 23.7   | 0.00001      |
| KEGG_PATHWAY                 | Transcriptional misregulation in cancer   | 8     | 21.1   | 0.000024      |
| KEGG_PATHWAY                 | Chemokine signaling pathway               | 8     | 21.1   | 0.000049      |
| KEGG_PATHWAY                 | PI3K-Akt signaling pathway                | 10    | 26.3   | 0.000064      |
| KEGG_PATHWAY                 | FoxO signaling pathway                    | 7     | 18.4   | 0.000069      |
Figure 6. The mark map of active components targets of Amydrium sinense on NF-kappa B signal pathway.

4. Conclusion
National medicine is similar to traditional Chinese medicine in a narrow sense, it has the characteristics of complex chemical composition, variety of clinical effects, and multi-component, multi-target and multi-channel coordination. The network pharmacology can provide a systematic method for ethnic medicine to discover the leading compounds, identify the targets, indications and analyze the relationship between the proteins. So that understand the mechanism of ethnic medicine more dynamically and holistically, when it used in disease prevention and control [20-21]. As a unique national medicine in China, Amydrium sinense, which explores the mechanism through the method of network pharmacology, it is a great significance for the development and research of national medicine [22]. It was firstly published in the “Outline of Xinhua materia medica”. It has a long history of clinical application and rich resources in China. The drug was widely used to treat rheumatism in ethnic areas of China. However, because the chemical compositions is numerous and complex, there are just few reports about its anti-inflammation. According to the reports, the treatment of rheumatoid arthritis is associated with regulate immune inflammation, and the rheumatoid arthritis is a kind of inflammatory disease. It's the main clinical manifestations are polyarthritis, symmetry and aggressive arthritis [23]. An important link in the pathogenesis of rheumatoid arthritis is due to the activation of T and B lymphocytes, a lot of inflammatory factors and autoantibodies are produced. It causes immune damage to the joints and organs. Inflammation is a preventive response by the occurrence of injury, stimulation or infection by organs and tissues. The specific performance is local red, swelling, heat, pain, functional disorders and often accompanied by pain [24]. It is also closely related to the occurrence and development of a variety of diseases, such as diabetes, heart disease, hypertension and other diseases [25-28]. The anti-inflammatory effect is also associated with the components of volatile
oil [29]. Many studies suggest that the mechanism of anti-inflammatory action may be correlated to the inhibition of early inflammatory telangiectasis and the reduction of capillary permeability [30-31] and the regulation of NF-kappa B signaling pathway [32]. Therefore, its pharmacological effects are mainly a combination of various pharmacological actions, it mainly contains inflammatory factors, inhibition and killing of pathogens, and nerve conduction to inhibit pain, etc.

In this study, the network pharmacology was used to search all the volatile oil components of Amydrium sinense by queried the literature. Selected 27 active chemical components and 404 active compounds corresponding to target points by the criteria with ADME parameters (OB ≥ 30% and DL ≥0.18) and pharmacodynamics activity. Therefore, the “component target disease” interaction network map was constructed. Through network topology parameter analysis, GO enrichment and KEGG pathway annotation analysis, 38 potential targets were screened, and 79 signaling pathways and 212 biological processes were involved in the anti-inflammatory effects of Amydrium sinense. The predicted results has showed that the action mechanism of Amydrium sinense volatile oil, it has a remarkable therapeutic effect on the treatment of inflammation. It is the anti-inflammatory active target distribution in different pathways, multi-component, multi-target coordination.

According to the anti-inflammatory direct-acting of target with topological parameter table, pathway and biological process; it should be emphasized that the anti-inflammatory effect is mostly closely related to the relevant regulated of NF-kB, TNF, Toll-like receptor signaling pathway, Hepatitis B and Inflammatory response, et al. NF-kappa B (NF-KB) is the common name of the recording factor family. It plays a dimer role and regulates the genes of immunity, inflammation and cell survival. It plays an important role in the inflammatory gene expression, which is induced in cellular signaling networks and the occurrence, development, outcome of inflammation [33]. In normal organism, the transcription activity of NF-KB is inhibited by I-kB. When the organism was subjected to strong stimulation, such as injury, infection, shock or poisoning, I-kB is degraded and loses its inhibition effect, and NF-KB was activated in vivo, it enhance NF-KB mediated inflammatory gene mRNA expression. Activation of NF KB signaling pathway by a large number of inflammatory factors. Such repeated circulation leads to tissue damage out of control. Therefore, NF KB is the key to regulate the expression of inflammatory factors [34-35]. In the NF-KB signal pathway diagram (Figure 4), it can be found that the main pathway leading to NF-KB activation is induced by tumour necrosis factor-alpha (TNF-alpha), interleukin-1 (IL-1), byproducts of bacterial and viral infections. Recent studies have found that [36] inflammatory factors can promote the increase of cyclooxygenase, which is TNFα and IL-1 produced in vivo, and it leads to a series of inflammatory reactions. In addition, COX-2 inhibitors have significant pain relief, it is caused by the relief of fever, headache and rheumatoid arthritis [37]. Modern studies have shown that the levels of NF-kB P65, TNFα, IL-1β and phosphorylated IκB-α in lung tissue of rats with acute lung injury were reduced, and the over-activation of nuclear factor kB/iκB signaling pathway was down-regulated, it was one of the volatile oil main mechanisms of anti-inflammatory effects [38]. This indicates that it may reduce the rheumatoid joint inflammation by down-regulating the NF-kB signaling pathway in inflammatory cells. Moreover, many studies have reported that [39] toll-like receptors play an important role in the initiation of inflammatory response and innate immunity, Hepatitis B indirectly regulates the inflammatory response of the body by inducing the stimulation of auxiliary T cells [40].

In this paper, based on the network pharmacology, the active components, target and mechanism of the volatile oil from Amydrium sinense were screened and analyzed. This study preliminarily revealed the role of the volatile oil from Amydrium sinense in the treatment of inflammation. These results indicate that the anti-inflammatory effect of Amydrium sinense has the characteristics of multi-component, multi-target and multi-channel. It provides theoretical basis for detection of the anti-inflammatory mechanism of Amydrium sinense, and provides a theoretical basis for its subsequent experiments.
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