Study on the mechanism of potential pharmacological action of Daphne Genkwa of anti-tumor based on data mining, network pharmacology and molecular docking

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Abstract. In this study, we explored the potential targets and mechanisms of Daphne genkwa anti-tumor through data mining, network pharmacology, and molecular docking techniques. The protein-protein Interaction Network (PPI) and the “active component-target” network of anti-tumor target sites of Daphne Genkwa were constructed, then the core target and the active components were studied by molecular docking. A total of 7 active ingredients, 116 potential targets, 9775 tumor-related targets were obtained. Among them, 177 were the intersection targets of ingredients and diseases. The ingredients-targets visual network diagram showed 126 nodes and 216 edges. GO and KEGG enrichment analysis showed the molecular functions, cellular ingredients, biological processes and pathways of the anti-tumor targets of Daphne genkwa. The results of molecular docking showed that the active components of Daphne Genkwa bind well to the anti-tumor core protein. In conclusion, Daphne genkwa can exert anti-tumor effects through multiple channels and targets.

1. Introduction
Tumor is a major disease that seriously threatens human health and life, and its incidence is increasing year by year. Although chemical drugs have improved the cure rate of many tumors, they also have strong side effects and drug resistance defects. Traditional Chinese medicine has a history of over 2000 years in the treatment of tumors\textsuperscript{[1]}. Modern pharmacological research has confirmed that many Traditional Chinese medicine herbs have sound curative effects in the treatment of tumors\textsuperscript{[2]}. The Daphne genkwa, belonging to the Marigold family, is widely distributed in the Yangtze and Yellow River basins of China. Daphne genkwa is a genus of Coriolus in the family Myrtleaceae and is widely distributed over the Yangtze and Yellow River basins of China. Modern pharmacological studies have proved that Daphne genkwa has a wide range of pharmacological effects, such as anti-tumor, anti-inflammatory, analgesic, sedative, anticonvulsant and immune system modulating biological activities, etc. In recent years, the study of the anti-tumor active ingredients of Daphne genkwa has become a hot spot, and researchers have done a lot of important work to elaborate the mechanism of the anti-tumor effect of Daphne genkwa\textsuperscript{[3]}. Flavonoids, diterpenoids, lignans and coumarins are the main anti-tumor
components in Daphne Genkwa. They have different degrees of anti-tumor effects, and the anti-tumor mechanism is still to be further studied by inhibiting cell proliferation, acting on cell cycle, promoting cell apoptosis and inhibiting protein synthesis. [5]
In this study, the active compounds and targets of Daphne genkwa were mined and screened by network pharmacology method, and the network relationship between active ingredients and anti-tumor targets of Daphne genkwa was constructed. The protein-protein interaction network relationship and GO and KEGG enrichment analysis were studied. The interaction between the core target and the active ingredients was verified by molecular docking. In this paper, the potential anti-tumor mechanisms of Daphne genkwa were explored at the molecular and protein levels to provide guidance for the development and clinical use of Daphne genkwa anti-tumor drugs.

2. Materials and methods

2.1. Active ingredients
The ingredients of Daphne genkwa were downloaded from the TCMSP database and screened for active ingredients with oral bioavailability (OB) $\geq 30\%$ and drug-like properties (DL) $\geq 0.18$. Also, the active ingredients of Daphne genkwa was supplemented with relevant literature.

2.2. Prediction of active ingredients targets
The active ingredient SMILES number was queried in the PubChem database and imported into the SwissTargetPrediction website to predict the active ingredients potential targets of Daphne genkwa.

2.3. Screening of tumor targets
Search the GeneCards database and OMIM database for the keyword "tumor" for tumor-related disease targets.

2.4. Constructing active ingredients-targets network relationships and screening core targets
The network relationships between active ingredients and potential targets were constructed using Cytoscape3.7.2 software, and Molecular docking of 4 high-value core targets was performed using the CytoNCA plug-in, and the the docking activity of the active component and the core anti-tumor target further were validated by AutoDock software.

2.5. Construction of Protein-Protein Interactions(PPI)Network
The anti-tumor targets of the active ingredients were imported into the String database website, and protein-protein interaction relationships were obtained with a confidence level (0.9) as the screening condition.

2.6. Enrichment Analysis
The anti-tumor targets of the active ingredients were imported into the Matescape database for analysis of GO enrichment (including GO biological processes, GO cellular ingredients, GO molecular functions and KEGG enrichment).

2.7. Molecular docking
Download the 2D structure files of the corresponding active components of four core genes from Pubchem database and convert them into 3D structures using Chem3D software as ligands. Download the 3D structures files of the proteins (AKT1, ESR1, PIK3R1, SRC) in the PDB database and remove the ligands and waters from the proteins by PyMOL software as the receptors. Molecular docking of the four core proteins to the active ingredients was simulated using AutoDock_Vina(1.1.2) software. PyMOL(2.5) software was used to visualize the molecular docking results.
3. Results and analysis

3.1. Active Ingredients of Daphne genkwa

A total of seven active ingredients of Coriandrum sativum were obtained through TCMSP database and literature screening, as shown in Table 1.

| Mol ID    | Molecule Name                                      | MW   | OB (%) | DL  |
|-----------|----------------------------------------------------|------|--------|-----|
| MOL001040 | (2 R)-5,7-dihydroxy-2-(4-hydroxyphenyl) chroman-4-one | 272.27 | 42.36  | 0.21 |
| MOL001506 | Supraene                                           | 410.8 | 33.55  | 0.42 |
| MOL000359 | sitosterol                                          | 414.79| 36.91  | 0.75 |
| MOL00422  | kaempferol                                          | 286.25| 41.88  | 0.24 |
| MOL005530 | Hydroxygenkwanin                                    | 300.28| 36.47  | 0.27 |
| MOL005573 | Genkwanin                                           | 284.28| 37.13  | 0.24 |
| MOL005574 | 9-Hydroxyglabratoide                                | 348.43| 104.66 | 0.32 |

3.2. Get the anti-tumor targets of Daphne genkwa

The active ingredient SMILLS number of Daphne genkwa was entered into the SwissTargetPrediction website to predict the active ingredient targets respectively. The targets were screened by probability ≥ 0.5, duplicate targets were removed, and finally 116 potential targets were obtained. The tumor targets were obtained by entering "tumor" in the GeneCard database and OMIM database respectively, and the targets in the two databases were combined and the duplicate targets were removed, resulting in 9775 relevant targets, and all the targets of the active ingredient and the tumor targets were intersected and made a Venn diagram to get 107 common targets, which were the anti-tumor targets of the active ingredient, as shown in Figure 1.

![Venn diagram](image)

Figure 1. Venn diagram of Daphne genkwa ingredients targets and tumor targets

3.3. Enrichment Analysis

The Go enrichment experiment showed that the 107 anti-tumor targets of effective components of Daphne Genkwa were closely related to lipid transport, lipid metabolism regulation, hormone level regulation, nuclear receptor activity and phosphatase binding. The result is shown in figure 2. The results of KEGG enrichment assay (Figure 3) showed that the 107 anti-tumor targets of effective components of Daphne Genkwa were closely related to the pathway of interaction of NRA receptor, TH17 cell differentiation, RAPI signal pathway and PPAR signal pathway.
3.4. Construction “Active Ingredients-targets” and “Targets-Pathways” Network

The “active ingredients-targets” network relationship diagram (Figure 4), and a "targets-pathways" network diagram (Figure 5) were built with Cytoscape3.7.2 software, where red nodes represented anti-tumor targets, blue nodes represented active ingredients, and purple represented pathways of KEGG. Figure 4 shows that there were 126 nodes and 261 edges, and Figure 5 shows that there were 74 nodes and 183 edges. The CytoNCA plug-in was used to screen the core targets and they were screened twice. Four core genes (5WBL, 3OS8, 3HHM and 2SRC) were obtained as receptors for molecular docking.

3.5. Construction of Protein-Protein Interactions Network

The protein-protein interactions network of the anti-tumor targets of Daphne genkwa was shown in Figure 6, and the degree values of each target were calculated and analyzed. The targets with values greater than the median were screened and the top 12 targets were selected for further analysis (Figure 7).
3.6. Molecular docking of active ingredients and core anti-tumor targets

To further validate the above analytical results, the simulation of molecular docking technology was used to explore the anti-tumor mechanism of Daphne genkwa active ingredients. The core targets obtained in Figure 7 were selected as receptors, including AKT1, ESR1, PIK3R1, and SRC. The molecular docking of the corresponding ingredients (5WBL, 3OS8, 3HHM, 2SRC) to the core targets were simulated by AutoDock Vina software, and protein activity pocket specific coordinate parameters and molecular docking results are shown in Table 2. The results showed that the active molecular ligands could have a good binding activity to the protein target receptors (Figure 8).

### Table 2. Parameters of ligand and receptor coordinates

| Receptor | Ligand | center_x | center_y | center_z | affinity |
|----------|--------|----------|----------|----------|----------|
| AKT1     | 5WBL   | -8.948   | 18.866   | -13.735  | -6.3     |
| ESR1     | 3OS8   | 4.473    | 38.959   | 8.937    | -8.6     |
| PIK3R1   | 3HHM   | 4.768    | 38.028   | 10.769   | -10.1    |
| SRC      | 2SRC   | 10.756   | 36.456   | 32.204   | -9.2     |

Figure 6. Schematic diagram of Protein-Protein interactions network of potential targets

Figure 7. Barplot diagram of the key targets for anti-tumor of active ingredients of Daphne genkwa

Figure 8. Docking of receptor proteins and active ingredients
4. Discussion

Research showed that China's cancer standardized incidence of 201.7 per 100,000, standardized mortality rate of 130.1 per 100,000 ranked 12th in the world[6]. Tumor is one of the most harmful diseases to human health at present. The most obvious feature of tumor is that it develops and metastasizes rapidly, and tumor is a chronic disease. With the in-depth research on Daphne genkwa, diterpene lactones, coumarins and flavonoids are the main anti-tumor compounds in Daphne genkwa, among which silymarin and hydroxysilymarin show obvious anti-tumor effects.

The core targets AKT1, ESR1, PIK3R1, and SRC in the protein-protein interaction network of Daphne genkwa anti-tumor proteins were further screened. The docking activity was also verified by molecular docking analysis. AKT1 is a highly conserved serine/threonine kinase and a key ingredient of the phosphatidylinositol 3-kinase (PI3K)/akt pathway. In addition, cell proliferation by AKT1 is promoted by cell cycle proteins such as p21, p27 and cyclinD1, while apoptosis is inhibited by p53[7-8].

It has been shown that anthocyanins enhance the expression of pAMPKα, thereby activating the AMPK protein kinase signaling pathway and inhibiting the mTORC2-H1993-mediated downstream signaling pathway. AKT1 has been identified in several research papers as a potential target for the treatment of cancer diseases[9]. ESR1 primarily encodes estrogen receptor protein A (ERα), which is expressed mainly in organs such as the uterus, ovaries and mammary glands. ERα can bind to estrogen (ERE) and then enzyme inhibitors form complexes on cells that affect receptor activity, thereby inhibiting gene transcription[10]. Many studies have shown that mutations in the ESR1 gene that activate the receptor without a ligand are associated with drug resistance in breast cancer therapy, and that patients with ESR1 mutations have poor prognosis, this makes ESR1 an important target for breast cancer treatment.

PIK3R1 is most common in ovarian cancer, and PIK3R1 deletion renders ovarian cancer cells susceptible to AKT or JAK2/STAT3 inhibition[11]. SRC family kinases (SFKs) play a key role in the pathogenesis of colorectal cancer (CRC), delivering SRC-mediated signals and facilitating cell migration[12]. SRC may activate sustained proliferation of tumor cells and invasion of normal cells.

5. Conclusion

In summary, this study investigated the potential mechanisms of Daphne genkwa in anti-tumor by network pharmacological approaches and molecular docking techniques. The results showed that Daphne genkwa can act through multiple pathways and targets to regulate gene transcription, immune regulation, selective killing of tumor cells and cell proliferation, thus achieving the effect of cancer prevention and treatment.

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