Original Article

Analyzing the multi-target pharmacological mechanism of folium *Artemisia argyi* acting on breast cancer: a network pharmacology approach

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Background: Folium *Artemisia argyi* (FAA) is a traditional Chinese herbal medicine that is widely used in the clinic. However, the underlying mechanisms of its anticancer effects have not been fully elucidated.

Methods: In this study, we applied a network pharmacology approach to identify the potential mechanisms of FAA against breast cancer. To be specific, we screened the active ingredients and potential targets of the FAA through the Traditional Chinese Medicine Systems Pharmacology (TCMSP) database. Meanwhile, we employed the oral bioavailability (OB) and drug-likeness (DL) to search for potential bioactive compounds of FAA. Breast cancer-related target genes data were gathered from the GeneCards and Online Mendelian Inheritance in Man (OMIM) databases, and the protein-protein interaction (PPI) data were acquired from the Search Tool for the Retrieval of Interacting Genes (STRING) database. In addition, we constructed the network and performed Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Enrichment Analysis.

Results: We obtained a total of nine active ingredients and 236 potential targets from FAA to construct a network, which showed that quercetin served as the major ingredient in FAA. *AKT1* (RAC-alpha serine/threonine-protein kinase), *MYC* (Myc proto-oncogene protein), *CASP3* (Caspase-3), *EGFR* (Epidermal growth factor receptor), *JUN* (Transcription factor AP-1), *CCND1* (G1/S-specific cyclin-D1), *VEGFA* (Vascular endothelial growth factor A), *ESR1* (Estrogen receptor), *MAPK1* (Mitogen-activated protein kinase 1), and *EGF* (pro-epidermal growth factor) were identified as key targets of FAA in the treatment of breast cancer. The PPI cluster demonstrated that *AKT1* was the seed in this cluster, indicating that *AKT1* played a crucial role in connecting other nodes in the PPI network. This enrichment demonstrated that FAA was highly related to signal transduction, endocrine system, replication and repair, as well as cell growth and death. The enrichment results also verified that the underlying mechanisms of FAA against breast cancer might be attributed to the coordinated regulation of several cancer-related pathways, such as the MAPK and mammalian target of rapamycin (mTOR) signaling pathways, among others.

Conclusions: This study identified the potential targets and pathways of FAA in the treatment of breast cancer using a network pharmacology approach, and systematically elucidated the mechanisms of FAA in the treatment of breast cancer.

Keywords: Folium *Artemisia argyi* (FAA); breast cancer; network pharmacology; herb

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Introduction

Breast cancer is the most frequent malignancy occurring in women with 1.38 million new cases each year and nearly 0.46 million related deaths globally (1,2). According to current projections, there will be approximately 3.2 million new cases per year by 2050 (3). Meanwhile, breast cancer is a kind of heterogeneous disease, with differences in occurrence, development, treatment, and prognosis (4). At present, comprehensive adjuvant treatments containing chemotherapy, radiotherapy, endocrine, and HER2-targeted therapies are widely used according to the five major molecular subtypes of breast cancer (5). However, these treatments are costly and usually result in a series of short- and long-term side effects, such as febrile neutropenia (6), alopecia (7), peripheral neuropathy (8) and cardiotoxicity (9), all of which significantly decrease the patient's quality of life. Furthermore, older patients in the terminal stages may also be more intolerant to these adverse reactions.

Folium *Artemisia argyi* (FAA), commonly called wormwood, is a perennial herb belonging to Artemisia in the Asteraceae family and rich in volatile oils, polysaccharides, flavonoids and other trace elements. FAA has strong adaptability and distributes in most parts of China. It is also cultivated in Mongolia, Korea, Russia's Far East and Japan. As a traditional Chinese herbal medicine, FAA has antipyretic, analgesic, and hemostatic effects (10). For thousands of years, it has been used internally to warm channels, arrest bleeding, dispel cold, and relieve pain, and is applied externally to eliminate dampness and relieve itching (11). Recently, owing to the various limitations of Western medicine, such as the toxicity and adverse side effects, increasing attention has been paid to the role of traditional Chinese medicine in the prevention and treatment of cancer (12). At the same time, Chinese herbs can target multiple points to achieve synergistic actions (13,14). According to pharmacology research, FAA contains multiple active chemical constituents, such as flavonoids, terpenoids, phenolic acids, and volatile oils (15,16), and exhibits a variety of effects, including anticancer, anti-inflammation, and anti-oxidation (17,18). For example, Shafi *et al.* suggested that FAA inhibited the proliferation and promoted apoptosis in breast cancer cells through Bcl-2 family proteins and the MEK/ERK pathway (10). It was also reported that FAA exhibited a dose-dependent inhibitory effect on hepatoma cells (11). However, although many studies have verified that FAA exerts remarkable antitumor functions, the underlying mechanisms have not yet been comprehensively understood (10,11,17,18).

It is widely known that herbal medicines include multi-component, multi-target, and multi-pathway features (19,20). Traditional Chinese medicine network pharmacology is a systematic research method based on the interaction network of herbs, compounds, targets, diseases, and genes (21). This approach emphasizes the integration of bioinformatics, systems biology, and pharmacology, which not only explains the complex interactions between herbs and diseases at a systematic level but also conforms to the systematic and holistic perspective of the traditional Chinese medicine theory (22,23). Thus, we utilized a network pharmacology approach in this study to explore the pharmacological mechanisms of FAA as a treatment for breast cancer. Firstly, we screened for active ingredients of FAA by estimating their oral bioavailability (OB) and drug-likeness (DL) (24). Next, we selected the common targets shared by the FAA compound targets and the breast

Highlight box

Key findings

- We explore the pharmacological mechanisms of FAA for breast cancer by a network pharmacology approach.

What is known and what is new?

- Many studies have verified that FAA exerts remarkable antitumor functions.
- The quercetin served as the major ingredient of FAA and might exert its anti-tumor effect mainly by acting on AKT pathway in breast cancer. The anti-tumor mechanism of FAA might be attributed to the coordinated regulation of several cancer-related pathways.

What is the implication, and what should change now?

- It provides a new theoretical basis and some new ideas for the studies of the treatment of breast cancer. It provides the feasibility of experimental research to study the mechanism of FAA in breast cancer, and then provides the possibility to find a new treatment strategy of breast cancer.

cancer-related targets using two databases [GeneCards and Online Mendelian Inheritance in Man (OMIM)] and then constructed the network by investigating the potential interactions between the various target nodes. In addition, the protein-protein interaction (PPI) data were obtained from the Search Tool for the Retrieval of Interacting Genes (STRING) database, and enrichment analyses [Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG)] were performed to explore the potential mechanisms of FAA against breast cancer. In summary, this study aimed to identify the potential targets and pathways of FAA as a treatment for breast cancer using the network pharmacology approach, and systematically elucidate the mechanisms of FAA in the treatment of breast cancer. We present the following article in accordance with the STREGA reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-5769/rc).

Methods

Data preparation

Active ingredients and targets against breast cancer in FAA

FAA ingredients were acquired from the Traditional Chinese Medicine Systems Pharmacology (TCMSP) database, which serves as a systematic platform to study herbs, including the identification of compounds and the screening of compound targets (25). In addition, to identify the corresponding targets of FAA compounds against breast cancer, the TCMSP database was utilized to identify potential targets. Finally, nine active herbal ingredients of FAA were selected (*Table 1*) by linking the active ingredients of FAA to the breast cancer targets. A total of 236 targets of FAA compounds were obtained in total (the specific targets are not shown).

Table 1 Active ingredients of FAA

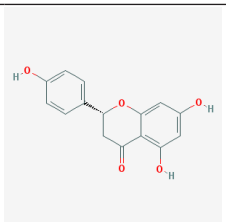
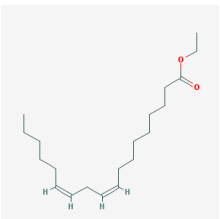
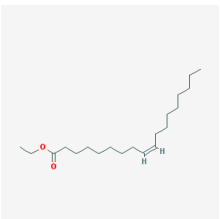
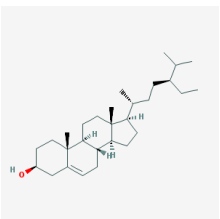
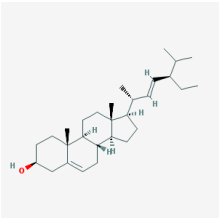
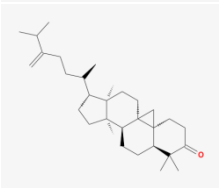
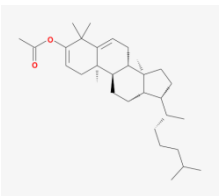
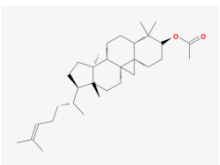
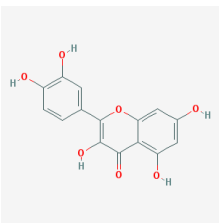
Mol ID	Mol name	2D structure	OB (%)	DL
MOL001040	(2R)-5,7-dihydroxy-2-(4-hydroxyphenyl) chroman-4-one		42.36	0.21
MOL001494	Mandenol		42	0.19
MOL002883	Ethyl oleate (NF)		32.4	0.19
MOL000358	beta-sitosterol		36.91	0.75

Table 1 (*continued*)

Table 1 (*continued*)

Mol ID	Mol name	2D structure	OB (%)	DL
MOL000449	Stigmasterol		43.83	0.76
MOL005720	24-methylenecyloartanone		41.11	0.79
MOL005735	dammaradienyl acetate		44.83	0.83
MOL005741	cycloartenol acetate		41.11	0.8
MOL000098	quercetin		46.43	0.28

FAA, folium *Artemisia argyi*; Mol, molecular; OB, oral bioavailability; DL, drug-likeness.

Pharmacokinetic predictions

In a pharmaceutical study, ADME (absorption, distribution, metabolism, and excretion) is a critical pattern to identify (24). Therefore, we employed two major ADME-related properties, namely, OB and DL to search for potential bioactive compounds of FAA. Ingredients with OB ≥30% and DL ≥0.18 were considered to be suggested drug screening criteria. The screening criteria of OB ≥30% and DL ≥0.18 to select ingredients was set based on previous studies (26,27). This criterion allows for more accurate screening of active ingredients. Detailed information on all of the ingredients before screening is listed in Table S1.

Potential target genes of breast cancer

Breast cancer-related target genes data were gathered from the GeneCards and OMIM databases. The species was set to Homo sapiens. GeneCards is an extensive platform that provides insight into predicted and annotated human genes. All of the gene-centric data were collected from 150 web resources, including genetic, genomic, proteomic, transcriptomic, and functional information (28). *Search strategy*: we set the keyword as "breast cancer" and the score30 after logging in to Genecards. The detailed information is listed in Table S2.

The OMIM is a comprehensive, authoritative, and

timely knowledgebase that links and catalogues all known diseases with a genetic component and provides further references to the genomic analyses of catalogued genes (29). Search strategy: we chose "gene map" on the website and then set the keyword as "breast cancer". The detailed information is listed in Table S3.

PPI data

We acquired the PPI data from the STRING database, which defines PPI with confidence ranges for data scores (high >0.7; medium >0.4; low >0.15) (30). In this study, we selected a confidence score of >0.4 to construct our PPI network.

Network construction

The PPI network has been widely applied to display many different interactions between proteins in the context of complex diseases (23,31), including breast cancer, prostate cancer, lung cancer, gastric cancer, etc. In this study, we constructed the network as follows: (I) we acquired the targets shared by the FAA compound targets and the breast cancer-related targets; (II) we entered these targets into the STRING database and obtained the FAA against breast cancer targets PPI network; and (III) we exported the PPI results as a simple tabular text output (.tsv) and then imported the .tsv file into Cytoscape (version 3.6.1, National Resource for Network Biology (NRNB), USA) to reconstruct the network to achieve better visualization and understanding for further analysis (32).

GO and KEGG pathway enrichment analysis

In this study, we used the Cluster Profiler package of R3.5.2 (Bioconductor, China) to perform GO enrichment analysis of the targets; the higher the score, the greater the importance of the genes represented in the list (33). The Cluster Profiler package of R3.5.2 was also used to analyze the KEGG pathway enrichment of overlapping target genes. KEGG analysis was used to explore the biological pathways and potential biological functions based on the enrichment analysis of functional items (34). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Results

Active ingredients

In this study, we acquired a total of nine active ingredients in FAA after ADME identification. Detailed information is shown in *Table 1* (all Mol IDs (Bioconductor, China) could be tracked in the TCMSP database). All of the FAA compounds before screening are presented in Table S1.

FAA compound-target network

To further uncover the potential pharmacological mechanisms of FAA against breast cancer, target genes common to both the active ingredients of FAA and breast cancer were selected in different databases. A total of 75 genes (Figure S1) belonging to both the FAA target gene and breast cancer target gene networks were screened via Venn analysis (*Figure 1A*). The compound-target network is presented in *Figure 1B* and includes 82 nodes and 171 edges, with a network density of 0.051 and a network diameter of 3. Detailed information on this network is depicted in *Table 2*.

PPI network

To explore the underlying mechanisms of FAA as a therapy against breast cancer, a PPI network of the FAA compound targets against breast cancer was constructed by connecting the targets of the FAA compounds and breast cancer. First, we obtained a total of 75 target genes belonging to both the FAA target gene and breast cancer target gene and obtained target symbol names using UniProt. Next, all of these 75 target genes were imported into the STRING database to generate the PPI results (settings: Homo sapiens and confidence>0.4). The original STRING PPI network is presented in Figure S2. Next, we imported the PPI data generated in the STRING database into Cytoscape (version 3.6.1).

As shown in *Figure 2A*, this PPI network included 75 nodes and 1,247 edges, with a network diameter of 3, a clustering coefficient of 0.733, and an average number of 33.253 neighbors. The average node degree was 33.3 (the degree was indicated both by the different colors and the size of the circles). Detailed information on this network is displayed in *Table 3*. All target degrees were calculated using this network. In *Figure 2B*, the 10 targets with the greatest degrees were *AKT1* (degree =67), *MYC* (degree =65), *CASP3* (degree =63), *EGFR* (degree =62), *JUN* (degree =61), *CCND1* (degree =60), *VEGFA* (degree =60), *ESR1* (degree =59), *MAPK1* (degree =57), and *EGF* (degree =55). As shown in *Figure 2C*, the cluster consisted of 68 nodes and 1,155 edges. The average node degree was 34 and the

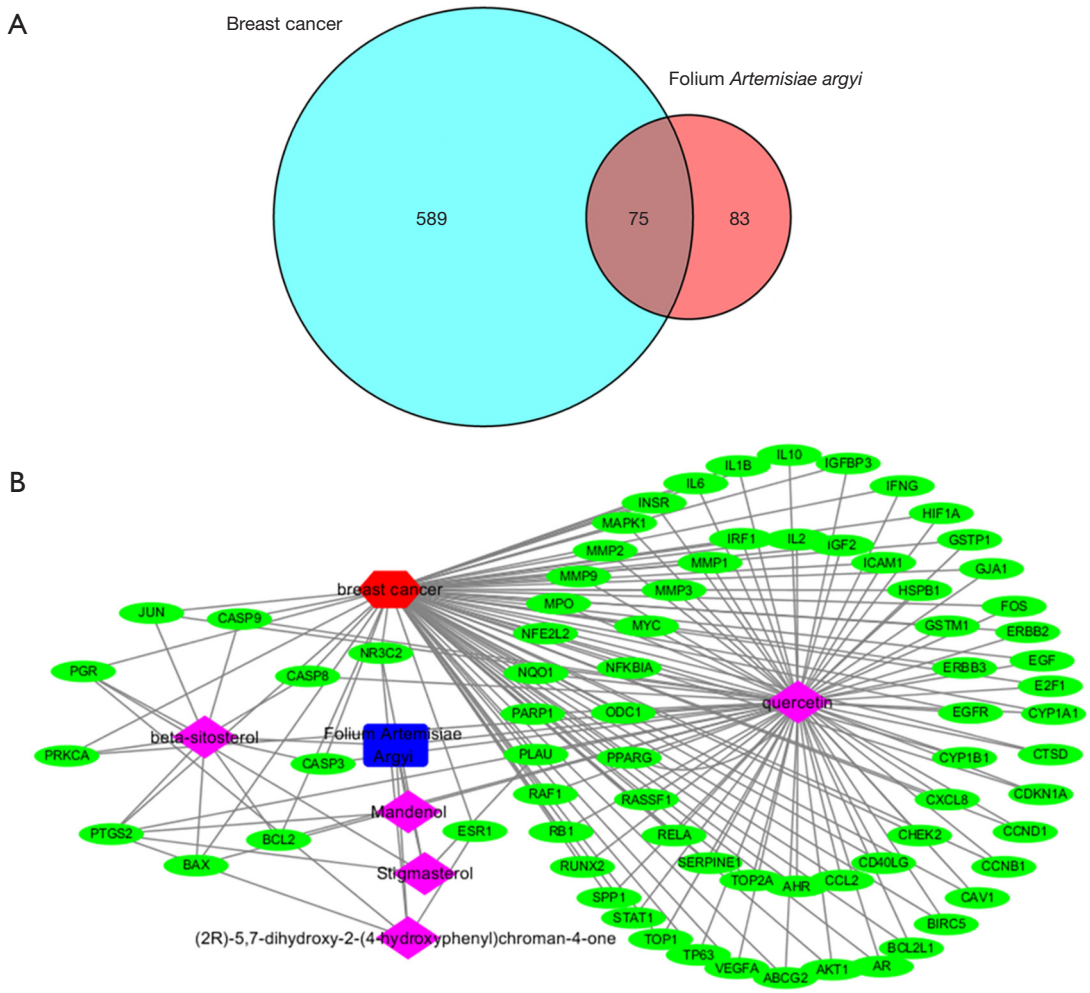


Figure 1 Seventy-five target genes for FAA against breast cancer. (A) Venn diagram showing the overlapping target genes for FAA against breast cancer. (B) Network of target genes for FAA against breast cancer. Pink diamond represents the active ingredients of FAA compounds; green ellipse represents the target genes of both FAA and breast cancer. FAA, folium *Artemisia argyi*.

Table 2 The FAA compound-candidate target network parameters

Network parameters	Values
Number of nodes	82
Network density	0.051
Network diameter	3
Network heterogeneity	2.678
Average number of neighbors	4.146
Characteristic path length	2.035
Shortest paths	6,642 (100%)
Network centralization	0.897

FAA, folium *Artemisia argyi*.

clustering coefficient was 0.77. *AKT1* (The red diamond in *Figure 2C*) was the seed in this cluster and interacted with the other FAA targets.

GO enrichment

To further discuss the multiple mechanisms of FAA as a treatment against breast cancer, we conducted GO enrichment analysis on the 75 common targets shared by the FAA compound targets and the breast cancer-related targets (35). Specifically, the top 30 targets are as follows (*Figure 2B*): *AKT1*, *MYC*, *CASP3*, *EGFR*, *JUN*, *CCND1*, *VEGFA*, *ESR1*, *MAPK1*, *EGF*, *IL6*, *PTGS2*, *ERBB2*, *FOS*, *MMP9*, *CXCL8*,

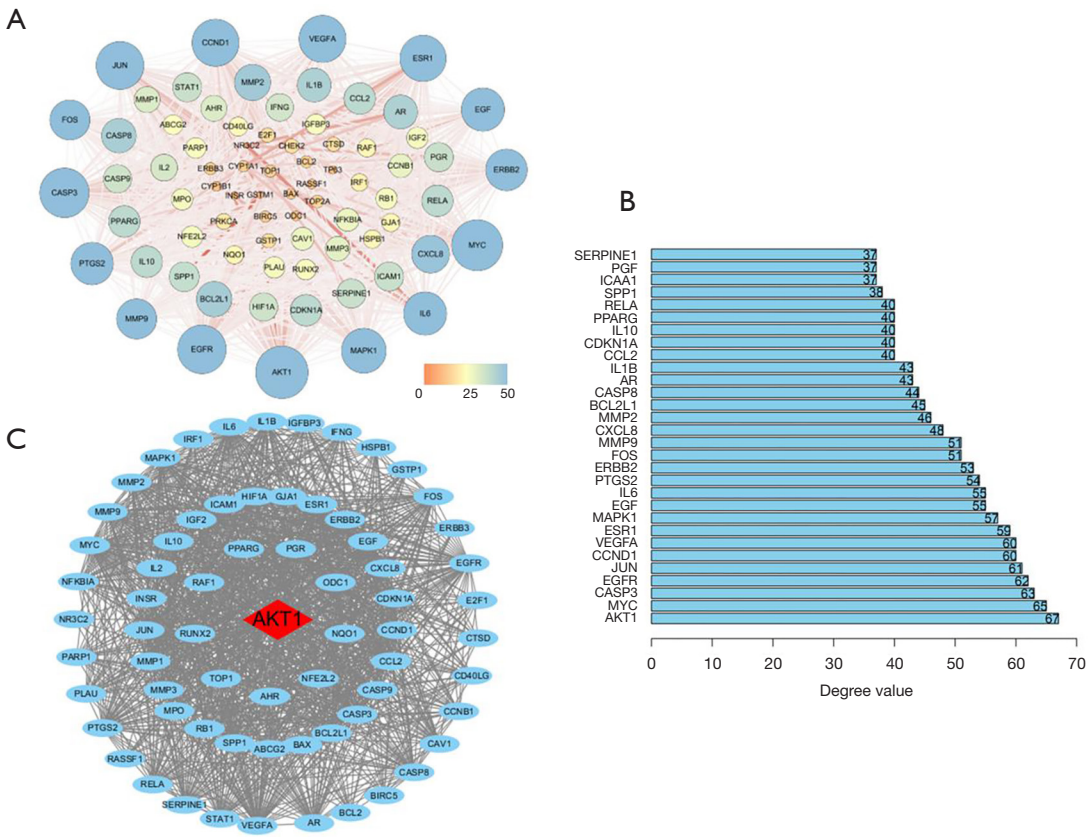


Figure 2 PPI network of FAA compound targets against breast cancer. (A) The PPI network was constructed using Cytoscape. Different colors represent the degree, as indicated by the scale. The size of the circle also indicates the degree. (B) The top 30 target genes of FAA against breast cancer screened by the PPI network. The X-axis represents degree values. (C) The cluster generated from (A). The red diamond in (C), AKT1, was the seed in this cluster and interacted with other FAA targets. PPI, protein-protein interaction; FAA, folium *Artemisia argyi*.

Table 3 The network parameters of FAA compound targets against breast cancer

Network parameters	Values
Number of nodes	75
Number of edges	1,247
Network diameter	3
Clustering coefficient	0.733
Average number of neighbors	33.253
Average node degree	33.3

FAA, folium *Artemisia argyi*.

MMP2, *BCL2L1*, *CASP8*, *AR*, *IL1B*, *CCL2*, *CDKN1A*, *IL10*, *PPARG*, *RELA*, *SPP1*, *ICAM1*, *PGR*, and *SERPINE1*. The significantly enriched GO targets are presented (adjusted P value <0.001) in *Figure 3*. The top five GO enrichment targets included (I) transcription factor activity, RNA polymerase II proximal promoter sequence-specific DNA binding (GO:0000982); (II) transcriptional activator activity, RNA polymerase II transcription regulatory region sequence-specific DNA binding (GO:0001228); (III) ubiquitin-like protein ligase binding (GO:0044389); (IV) cytokine receptor binding (GO:0005126); and (V) transcriptional activator activity, RNA polymerase II proximal promoter sequence-specific DNA binding (GO:0001077). Detailed

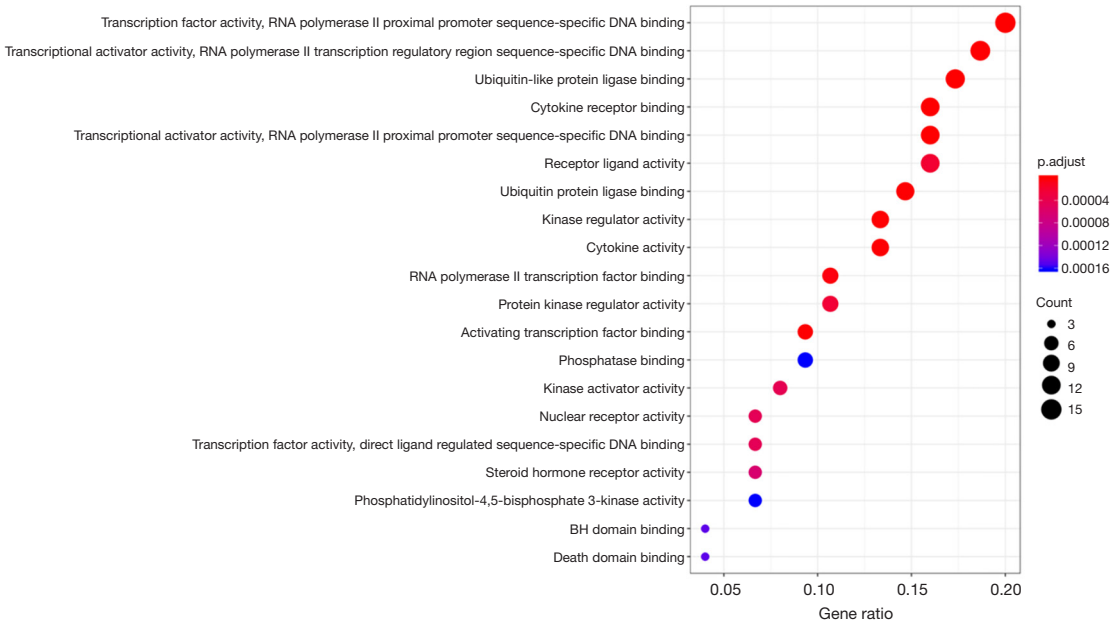


Figure 3 GO enrichment analysis of the 75 common targets shared by the FAA compound targets and breast cancer-related targets. The color represents the different adjusted P values, while the size of the circle represents the count. GO, Gene Ontology; FAA, folium *Artemisia argyi*.

GO enrichment information was shown in *Table 4*. Thus, we speculated that FAA probably executed its pharmacological effects on breast cancer by simultaneously involving these molecular functions.

KEGG enrichment

We obtained a total of 74 pathways belonging to several categories, including human diseases, cellular processes, and drug resistance, among others. Among these, the top 30 significantly enriched KEGG targets are presented (adjusted P value <0.001) in *Figure 4*. In the cancer-related disease, prostate cancer (hsa05215), bladder cancer (hsa05219), pancreatic cancer (hsa05212), breast cancer (hsa05224), colorectal cancer (hsa05210), non-small cell lung cancer (hsa05223), small cell lung cancer (hsa05222), gastric cancer (hsa05226), endometrial cancer (hsa05226), renal cell carcinoma (hsa05211), thyroid cancer (hsa05216), and small cell lung cancer (hsa05222) data were processed using KEGG enrichment analysis. Detailed KEGG information is shown in *Table 5*.

Discussion

As demonstrated in *Figure 1B*, quercetin was the most critical component of FAA, which was connected to the most targets. It is a flavonoid found in natural plants and exhibits a variety of activities such as antioxidant, anti-inflammatory, antiviral, and antimicrobial effects through multiple signal transduction pathways (35,36). Several studies have validated that quercetin can inhibit the progression of various tumors, including breast cancer (37), prostate cancer (38), gastric cancer (39), ovarian cancer (40), and colorectal cancer (41). Moreover, some studies have also reported that it not only has a synergistic effect when combined with chemotherapeutic or radiotherapy agents but can also mitigate the expected adverse side effects and toxic reactions (42,43).

In addition, our results showed that numerous targets were affected by two or more compounds. For instance, Prostaglandin G/H synthase 1 (*PTGS1*) and Prostaglandin G/H synthase 2 (*PTGS2*) were both modulated by quercetin, stigmasterol, mandenol, etc. Constitutive *PTGS1* and inducible *PTGS2* belong to two isozymes of *PTGS*, which have pivotal effects both as a peroxidase and a dioxygenase (44). Other studies have suggested that *PTGS2* might inversely control the metastasis and chemoresistance of breast cancer via the regulation of *EMT* (Epithelial-mesenchymal transition), apoptosis, and senescence (45-47). Also, nuclear receptor coactivator 2 (*NCOA2*), a member

Table 4 GO enrichment results

ID	Description	Count	Adjust P value
GO:0000982	Transcription factor activity, RNA polymerase II proximal promoter sequence-specific DNA binding	15	<0.0001
GO:0001228	Transcriptional activator activity, RNA polymerase II transcription regulatory region sequence-specific DNA binding	14	<0.0001
GO:0044389	Ubiquitin-like protein ligase binding	13	<0.0001
GO:0005126	Cytokine receptor binding	12	<0.0001
GO:0001077	Transcriptional activator activity, RNA polymerase II proximal promoter sequence-specific DNA binding	12	<0.0001
GO:0048018	Receptor ligand activity	12	<0.0001
GO:0031625	Ubiquitin protein ligase binding	11	<0.0001
GO:0019207	Kinase regulator activity	10	<0.0001
GO:0005125	Cytokine activity	10	<0.0001
GO:0001085	RNA polymerase II transcription factor binding	8	<0.0001
GO:0019887	Protein kinase regulator activity	8	<0.0001
GO:0033613	Activating transcription factor binding	7	<0.0001
GO:0019902	Phosphatase binding	7	0.0002
GO:0019209	Kinase activator activity	6	<0.0001
GO:0004879	Nuclear receptor activity	5	<0.0001
GO:0098531	Transcription factor activity, direct ligand regulated sequence-specific DNA binding	5	<0.0001
GO:0003707	Steroid hormone receptor activity	5	<0.0001
GO:0046934	Phosphatidylinositol-4,5-bisphosphate 3-kinase activity	5	0.0002
GO:0051400	BH domain binding	3	0.0001
GO:0070513	Death domain binding	3	0.0001

GO, Gene Ontology.

of the p160 family, performs key roles in many different physiological and pathological processes, including cell growth, energy metabolism, endocrine regulation, and circadian rhythms (48). More importantly, *NCOA2* gene expression plays crucial roles in the development, progression, and metastasis of malignant tumors, including breast cancer (49). In prostate cancer patients, the high expression of *NCOA2* is more likely to relapse after androgen deprivation therapy (50).

Similarly, Beta-2 adrenergic receptor (*ADRB2*), Gamma-aminobutyric acid receptor subunit alpha-1 (*GABRA1*), Heat shock protein HSP 90, Progesterone receptor (*PGR*), and Sodium channel protein type 5 subunit alpha (*SCN5A*) could also be regulated by more than two active ingredients. In this study, we obtained an approximate observation of the relationship between these active ingredients and targets and also discovered the potential pharmacological effects of FAA from this network (*Figure 1B*).

Figure 2C intuitively indicated that *AKT1* played an important role in connecting other nodes in this PPI network. It is well-known that the serine/threonine kinase, *AKT1*, one of the three isoforms of the Akt family, emerged as a downstream effector of *PI3K* (51). *AKT* inhibits apoptosis by suppressing the actions of BAD (BCL2 Associated Agonist of Cell Death) and caspase-9 (52). In breast cancer, *AKT1* activation accelerates cell proliferation, while Akt1 inhibition promotes epithelial-to-mesenchymal transition (53).

Detailed GO enrichment information is shown in *Table 4*. We speculated that FAA probably executed its

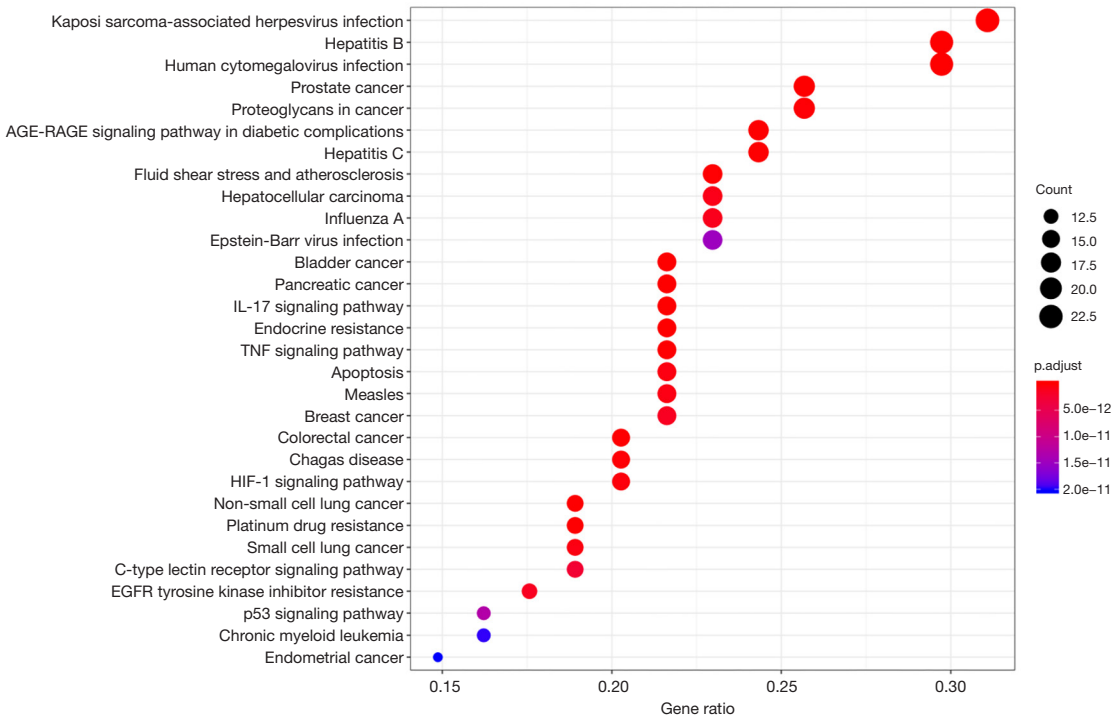


Figure 4 KEGG enrichment analysis of the 75 common targets shared by the FAA compound targets and breast cancer-related targets. The color represents the different adjusted P value, while the size of circle represents the count. KEGG, Kyoto Encyclopedia of Genes and Genomes; FAA, folium *Artemisia argyi*.

pharmacological effects on breast cancer by simultaneously involving these molecular functions. We further carried out KEGG (34) enrichment analysis on 75 common targets to clarify the integral regulation of FAA in the treatment of breast cancer. The results (shown in *Table 5*) indicated that FAA has a significant potential to treat a wide range of cancers, such as breast cancer (10), prostate cancer (54), bladder cancer (55), colorectal cancer (56), and gastric cancer (57), which is consistent with previous research. Furthermore, the results also verified that these signaling pathways remarkably enriched by potential targets of FAA in breast cancer were strongly associated with signal transduction, endocrine system, replication, repair, as well as cell growth and death, most of which played an essential role in the development and progression of cancers, such as the PI3K/AKT signaling pathway (hsa04151) (58), the MAPK signaling pathway (hsa04010) (59), the mammalian target of rapamycin (mTOR) signaling pathway (hsa04150) (60), apoptosis (hsa04210), and the cell cycle signaling pathway (hsa04110) (61). Therefore, we speculated that the underlying mechanism of FAA against breast cancer might be attributed to the coordinated regulation of several cancer-related pathways. About 30% of breast cancer patients who have poor prognosis show overexpression and amplification of *HER2* gene. The high receptor concentration on the membranes of HER2 overexpressing cells can activate the PI3K/AKT and the RAS/RAF/MAPK pathways, which initiates cell proliferation, growth, survival, invasion and angiogenesis. It is considered that FAA is related to the above signaling pathways in breast cancer. FAA may inhibit the recurrence and metastasis of HER2-positive breast cancer patients, which needs further verification (62,63).

Although breast cancer patients get better after various treatments, most patients still have drug resistance and show disease progression. This process involves multiple signaling pathways. According to our results, FAA may be related to multiple signaling pathways in breast cancer. Quercetin was the most critical component of FAA, several studies have validated that quercetin can inhibit the progression of various tumors (38,41,42). Therefore, FAA may be considered as a therapeutic agent against various cancers including breast cancer in the future. The absence of experimental data is indeed the weakness of this

Table 5 KEGG enrichment results

ID	Description	Count	Adjust P value
hsa05167	Kaposi sarcoma-associated herpes virus infection	23	<0.0001
hsa05161	Hepatitis B	22	<0.0001
hsa05163	Human cytomegalovirus infection	22	<0.0001
hsa04151	PI3K-Akt signaling pathway	21	<0.0001
hsa05215	Prostate cancer	19	<0.0001
hsa05205	Proteoglycans in cancer	19	<0.0001
hsa05206	MicroRNAs in cancer	19	<0.0001
hsa04933	AGE-RAGE signaling pathway in diabetic complications	18	<0.0001
hsa05160	Hepatitis C	18	<0.0001
hsa04010	MAPK signaling pathway	18	<0.0001
hsa05165	Human papillomavirus infection	18	<0.0001
hsa05418	Fluid shear stress and atherosclerosis	17	<0.0001
hsa05225	Hepatocellular carcinoma	17	<0.0001
hsa05164	Influenza A	17	<0.0001
hsa05169	Epstein-Barr virus infection	17	<0.0001
hsa05166	Human T-cell leukemia virus 1 infection	17	<0.0001
hsa05219	Bladder cancer	16	<0.0001
hsa05212	Pancreatic cancer	16	<0.0001
hsa04657	IL-17 signaling pathway	16	<0.0001
hsa01522	Endocrine resistance	16	<0.0001
hsa04668	TNF signaling pathway	16	<0.0001
hsa04210	Apoptosis	16	<0.0001
hsa05162	Measles	16	<0.0001
hsa05224	Breast cancer	16	<0.0001
hsa05210	Colorectal cancer	15	<0.0001
hsa05142	Chagas disease	15	<0.0001
hsa04066	HIF-1 signaling pathway	15	<0.0001
hsa04218	Cellular senescence	15	<0.0001
hsa05152	Tuberculosis	15	<0.0001
hsa05170	Human immunodeficiency virus 1 infection	15	<0.0001

KEGG, Kyoto Encyclopedia of Genes and Genomes.

study. In fact, the relevant experiments are carrying out to validate the representative active ingredients of FAA, but have not been completed. We are very pleased to report the experimental results in the subsequent studies.

Conclusions

At present, although many studies have verified that FAA exhibits arresting antitumor activities, the underlying mechanisms of its antitumor activities have not yet been

fully elucidated. Network pharmacology emphasizes the integration of bioinformatics, systems biology, and pharmacology, which not only explain the complex interactions between diseases and Chinese herbs at a systematic level but also conform to the systematic and holistic perspective of traditional Chinese medicine theory (12). To better explore the pharmacological mechanisms of FAA as a treatment for breast cancer, we applied the network pharmacology approach to identify the potential mechanisms of FAA as a breast cancer treatment by compound-target network construction, PPI network, and GO and KEGG enrichment analyses. We took advantage of OB and DL to explore the potential active ingredients of FAA. At present, there are few studies on the pharmacokinetics of FAA. Choi *et al*. found that FAA exerted anticancer activities through the inhibition of cell growth and the induction of apoptosis in breast cancer cells (17).

In this study, we acquired nine active ingredients and 236 potential targets from FAA and validated a synergistic herb strategy featuring multi-component, multi-target, and multi-pathway characteristics. The compound-target network confirmed that quercetin served as the major ingredient in FAA. Moreover, the PPI network provided information concerning the source of the interactions. PPI analysis indicated that FAA had a significant effect on breast cancer by influencing the whole biological network, including targets such as *AKT1*, *MYC*, *CASP3*, *EGFR*, *JUN*, *CCND1*, *VEGFA*, *ESR1*, *MAPK1*, and *EGF*. The PPI cluster demonstrated that *AKT1* was the seed, suggesting that *AKT1* played a crucial role in connecting other nodes in the PPI network. Next, enrichment analysis indicated that FAA was strongly related to signal transduction, the endocrine system, replication and repair, and cell growth and death. The enrichment results also showed that the underlying mechanism of FAA against breast cancer might be attributed to the coordinated regulation of several cancer-related pathways, such as the MAPK and mTOR signaling pathways, among others.

In conclusion, this study applied a network approach demonstrating how FAA compounds alter different pathways against breast cancer, which was supplementary to other studies on drugs against breast cancer. Furthermore, we confirmed that FAA substantially influenced numerous breast cancer-related targets, a finding that was consistent with present cancer study trends showing that the occurrence and development of breast cancer is a result of the gradual accumulation of distinct genome modifications in cancer cells (64,65). We fully expect that our research can help to promote the employment of network pharmacology in uncovering the potential mechanisms of anticancer Chinese herbs and provide clues to assess the synergy of herbs in the treatment of other complex diseases, especially cancer.

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Footnote

Reporting Checklist: The authors have completed the STREGA reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-5769/rc

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-5769/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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