Active mechanism of "Zishen Huayu Fang" in treating intrauterine adhesions based on network pharmacology and macromolecular docking

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Abstract: The study is investigating the mechanism of therapeutics of "Zishen Huayu Fang" in the adhesions of intrauterine. Pharmacology systems have been employed as active ingredients of 131 compounds like Luteolin, Quercetin, and Kaempferol in this research study in a combination of other compounds to prove the efficacy of the Zishen Huayu Fang in the treatment of adhesions inside the uterus. The study aims to understand how the ingredients in "Zishen Huayu Fang" interact with the body, particularly how they can assist in the prevention, mitigation and elimination of adhesions inside the uterus. KEGG and GO databases and tools are used to analyze gene functions and interactions in the proposed research study. The signaling pathways such as PI3K-Akt, AGE-RAGE, HIF-1, TNF, are involved in the study to understand the critical aspects of cellular function, such as inflammation, metabolism and cell survival. This study is examining how the formula affects these crucial pathways, which could be linked to its therapeutic effects on uterine adhesions. The study suggests that "Zishen Huayu Fang" influences the expression of key targets, particularly STAT3, MAPK1, AKT1, HSP90AA1 and JUN, through active ingredients, providing a foundation for further investigation into its therapeutic efficacy in intrauterine adhesions.

Keywords: Macromolecular docking, network pharmacology, Zishen Huayu Fang, uterine adhesions.

INTRODUCTION

Intrauterine adhesions (IUA) are common gynecological diseases that cause female damage of reproductive function in women. The clinical manifestations include decreased menstruation, even amenorrhea, recurrent miscarriage, dysmenorrhea and infertility (Bai *et al*., 2021). Currently, it is widely believed that trauma and infections are the two main causes of IUA. Besides, the incidences of IUA that is influenced by multiple induced curettage and abortions with high range of 25% -30% (Chen *et al*., 2023) and the uterine cavity surgeries are rising every year at a rapid rate. The preferred treatment for IUA is transcervical resection of adhesions (TCRA) under hysteroscopy (Dang *et al*., 2022). However, secondary damage to the endometrium is unavoidable since it is uterine cavity operation, which seriously affects women's reproduction system and mental health as well. Therefore, it has gradually become a focus to explore effective and safer methods to prevent and treat intrauterine adhesions in reproductive medical science. Traditional Chinese medicines show many advantages in preventing and curing intrauterine adhesions and recurrence of TCRA postoperative adhesions, for improving clinical symptoms, and increasing fertility rate (Du *et al*., 2020). The clinical efficacy of "Zishen Huayu Fang" is significant while its specific acting mechanism is still unclear. Therefore, this paper, through pharmacology of network and docking the Macromolecular, attempts to

explore the ingredients that impact the treatment of intrauterine adhesions using "Zishen Huayu Fang". In addition, it has initially revealed its possible targets and mechanisms to provide some reference for its subsequent clinical applications and futuristic research for dealing with multiple ailments in human body (Gao *et al*., 2022).

The idea presented by the authors (Xu Y, 2023) is based on Taoist philosophy. The author demonstrates that the healthy life is connected with the ways of nature of universe. Health and illness are caused by an imbalance of the two basic forces, yin and yang and by the influence of the five elements (water, fire, metal, wood and earth) on the organs of the body. The organs themselves were thought to interact in ways that seem physiologically strange nowadays. Most of the traditional Chinese medicines follow the principles stated in this book.

Uterine adhesions can be diagnosed through pelvic exams, ultrasounds, hysterosalpingography (HSG), or hysteroscopy. The symptoms can include irregular menstrual cycle, Amenorrhea, discomfort or pelvic pain, and fertility issues as well. The uterine adhesions can be classified as "amenorrhea" and "menorrhagia" according to clinical symptoms. Herbal medicine exhibits the history of long treating menstrual diseases and infertility (Jiang *et al*., 2021). Modern TCM scholars believe that the uterine cavity operation directly damages the uterus and Chongren, resulting in abnormal regulation of the kidney-Tiangui -Chongren-uterine axis. These damages resulting from uterine cavity causes the vitality and blood essence

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of the kidney, resulting in emptiness of the blood sea of Chongren and even amenorrhea (Guo *et al*., 2024). Moreover, the blood supply is also depleted after the operation of the uterine cavity. Therefore, external pathogens are prone to invade by taking advantage of the deficiency, forming blood stasis with the blood pulse, and blood stasis obstructs the uterus, causing various symptoms (Huang *et al*., 2023). On the top of it, after the operation of the uterine cavity, the external bacteria and virus can easily invade by taking advantage of the deficiency and fight with the blood to form stasis, and the stagnant blood will block the uterus internally and cause all the symptoms (Huang *et al*., 2024). Kidney deficiency and blood stasis is the basic pathogenesis of postoperative uterine adhesions. Therefore, the basic treatment should be designed to nourish the kidney and invigorate blood, resolve blood stasis and regulate menstruation. In the procedure for nourishing the kidney and removing blood stasis, *Rehmannia glutinosa, Cuscuta chinensis, Rubus idaeus, Polygonatum sibiricum* Red etc. nourish the kidney and fill the kidney essence. In the formula for nourishing the kidney and resolving blood stasis, rehmanniae, codonopsis, raspberry and yellow essence nourish kidney Yin and fill the kidney essence whereas the tortoise nails nourish the yin (a fluid in Chinese medicine that nourishes kidney) and antler slices warm Yang (a concept in Chinese medicine where kidney energy is stored), both of which are sentient products of flesh and blood (Li *et al*., 2023). Yang gets the help of Yin to grow and the source is inexhaustible, Yin is part of Yang and both help each other in replenishing kidney health. Chuanxiong, *Angelica sinensis, Salvia miltiorrhiza*, tangerine peel, red peony and Puhuang promote circulation and stagnation to facilitate circulation of blood and disperse blood stasis. Chicken blood wine, Red wine, *Portulaca oleracea* and *Sophora Sinensis* can clear heat, promote blood circulation to remove meridian obstruction after uterine cavity (Niu *et al*., 2021). The entire procedure adopted by Chinese medicine tackles the problem; the yin and yang are complementary to each other to nourish the kidney and fill the essence and invigorate the blood as well as remove blood stasis (Liu *et al*., 2024). Luteolin is an antioxidant and anti-inflammatory agent that can also boost the activity of Superoxide dismutase (SOD) and enhance the body's antioxidant capacity, reduce oxidative damage and can promote the development of immune organs and the differentiation of T lymphocytes (Meng *et al*., 2023); Kaempferol can alleviate the inflammatory response by regulating related signaling pathways of nuclear transcription factors κB (nuclear factor-κB, NF-κB) and inhibit inflammatory factors, including tumor necrosis factor-α (tumor necrosis factor-α, TNF-α), IL-1β、IL-6. It can also reduce hypertrophic scars by effectively inhibiting excessive proliferation of fibroblasts and excessive deposition of extra cellular matrix by down regulating miR-21(Mengting *et al*., 2022).

This paper has the following contributions
- The paper is highlighting the advantages of the traditional Chinese medicine "Zishen Huayu Fang" in treating intrauterine adhesions based on network pharmacology and macromolecular docking.
- A comprehensive methodology is devised to show the use of "Zishen Huayu Fang" in treating intrauterine adhesions.
- The results are derived by considering multiple parameters to show the efficacy of "Zishen Huayu Fang" in treating intrauterine adhesions.
- The outcome of the proposed study reveals that the Chinese medicine is effective in the treatment of intrauterine adhesions

MATERIALS AND METHODS

Active ingredients screening and targets by "Zishen Huayu Fang"
The authentic database is exploited to prove the utilization of traditional Chinese Medicine (TCMSP, https://tcmspw.com/tcmsp.php) in treating uterus adhesions. The targets are subjects suffering from uterus adhesions and active ingredients used in CTM are Radix Rehmanniae Preparata, Semen Cuscutae, Rubus idaeus, Rhizoma Polygonati Rhizoma, Rhizoma Chuanxiong, Pericarpium Citri Reticulate, *Angelica sinensis*, Radix Paeoniae Rubra, Radix Salviae Miltiorrhizae, Herba Typhae, Caulis Spatholobi, Red Rattan, *Portulaca oleracea* and *Gleditsia sinensis* for the treatment of uterine adhesions. The components and targets of turtle shell and deer antler tablets were identified using the molecular mechanism bioinformatics analysis tool for traditional Chinese medicine, known as Batman-TCM (available at http://bionet.ncpsb.org.cn/batman-tcm/index.php/Home/Index/index). High-throughput experiments and reference databases for herbal medicine, such as HERB (http://herb.ac.cn/), were used in this study because deer antler and turtle shell tablets are not included in the TCMSP database for traditional Chinese medicine. A substance to be considered for further study and development, it must meet the following two criteria:

1. *Oral Bioavailability (OB) > 30%*: At least 30% of the substance must be absorbed into the bloodstream when taken orally that means it should be effectively utilized by the body.
2. *Drug likeness (DL) ≥ 0.18*: The substance must have a drug likeness score of 0.18 or higher, and it is suggesting that the treatment possesses characteristics similar to known drugs, which could indicate its potential as an effective medication.

The above criteria help in finding whether a compound is suitable for consideration as a therapeutic agent
All targets have been verified using the UniProt database (https://www.uniprot.org/) to remove any duplicates and

non-human targets. This ensures that only unique and relevant targets from human sources are included in the analysis.

Target screening for intrauterine adhesions

The human gene database (GeneCards, https://www.genecards.org/), the NCBI gene database (https://www.genecards.org/) and the DisGeNET database (https://www.genecards.org/) were utilized to search for the targets of intrauterine adhesion disease, which were then consolidated and censored to obtain the desired gene targets related to intrauterine adhesions.

Constructing the PPI (protein-protein interaction) network to treat intrauterine adhesions by "Zishen Huayu Fang"

PPI networks play a crucial role in finding the key proteins involved in the biological pathways related to intrauterine adhesions. By analyzing these interactions, we can diagnose which proteins are critical to the condition. By investing how these proteins interact within the networks, it could be analyzed which molecular mechanisms were behind intrauterine adhesions and how Zishen Huayu Fang might have influence these processes.

The networks with the aid of PPI can validate potential gene or protein targets that Zishen Huayu Fang may affect, and enhance the understanding of its therapeutic effects. PPI networks can lead to more personalized treatment approaches by identifying specific targets that are relevant to individual patients' conditions. PPI networks provide a framework for understanding the complex biological interactions involved in intrauterine adhesions and how herbal therapies like Zishen Huayu Fang can be utilized effectively.

Venny 2.1 software was utilized for a Venny diagram to gain drug disease intersection targets, which were imported through the https://string-db.org/cgi/input.pl String database. With a dependability of more than 0.9, "Homo sapiens" is identified as the biological species. Ultimately, a network of protein-protein interactions (PPIs) was obtained.

Network construction of "Zishen Huayu Fang" drugs-Active ingredient

The active ingredients in "Zishen Huayu Fang" have been identified and chosen on the basis of their potential impacts on uterine adhesions. Targets associated with uterine adhesions have also been identified by using the existing research and databases. Then, by utilizing this gathered information and after screening the active components, uterine adhesion and action targets, a network diagram was formed in Cytoscape 3.8.0.

Analysis of enrichmentt of GO and enrichmentt of the pathway of KEGG

The common targets associated with the biological processes have been analyzed through various steps to get knowledge about their functions. Gene Ontology (GO) analysis focuses on three different categories such as Molecular Function (MF) which is used in identifying what the proteins or enzymes do at the molecular level, Biological Process (BP) is exploited in understanding the biological activities which are caused by these proteins, and Cell Component (CC) is used to determine the location where these proteins are located in the cells.

Pathway enrichment analysis uses the Kyoto Encyclopedia of Genes and Genomes (KEGG) database to find out the specific biological pathways where the common targets are involved in. In pathway analysis, the string database is utilized to ensure that the findings meet the statistical significance criteria, with a p-value < 0.05 as threshold for screening.

To represent the findings visually, R software (version 4.0.3) has been employed. ggplot2 is used for creating customizable visualizations. clusterProfiler is used for visualizations of genes to check their functional profiles. Bubble charts are also used to provide a visual summary of the enrichment analysis.

Preparation of proteins

The analysis of proteins is significant in biological pathways. The Proteins are required for all the biological functions. The proteins act as enzymes, signalling molecules, structural components, and transporters. The proteins involved in pathways such as PI3K- HIF-1, Akt, TNF, AGE-RAGE and IL-17 play their roles in cellular processes important to health and disease identification.

The pathways mentioned in this research study which are critical for various cellular responses, include survival, growth, immune responses and inflammation. PI3K-Akt is involved in cell growth. AGE-RAGE is playing important role in inflammation and aging. HIF-1 controls reactions to low oxygen levels. TNF and IL-17 are the cytokines in inflammatory responses. By paying attention on these key proteins within these pathways, the physicians can identify the potential therapeutic targets. It is very important to identify the proteins associated with particular disease progression.

Preparation of ligands

The structures of the flavonoids (Quercetin, Kaempferol and Luteolin) were methodically prepared for docking simulations through different key steps. This included adjusting their molecular structures to reflect stable conformations and assessing their protonation states based on the biological environment. This thorough preparation was aimed to explore how these flavonoids interact with particular proteins and how these proteins can potential help in treating intrauterine adhesions.

Macromolecular docking

The molecular docking is a computational method that predicts how small molecules bind to a target protein. The

motive is to determine the interactions between the proteins for a drug design. Getting the structures of key proteins from protein databases is critical for accurate modeling. Preparing these proteins for docking helps in visualizing and understanding how different compounds, such as those found in "Zishen Huayu Fang," may influence the pathways involved in intrauterine adhesions.

Core genes and the primary active components of "Zishen Huayu Fang" were paired via macromolecular docking. From PDB data, the relevant target protein structure was located and downloaded. The program PyMOL 1.7.2 was used to alter the target protein's structure. The primary active components' 2D structures were obtained and added to the PubChem database. Next, small molecule compounds having 2D structures were converted into 3D structures in mol2 format using Open Babel 2.4.1 software. Additionally, the software AutoDock Tools 1.5.6 was used to combine non-polar hydrogen, determine the charge and dehydrate and hydrogenate target proteins and small molecule compounds. The software Autodock Vina 1.1.2 was utilized for Macromolecular docking.

KEGG (Kyoto Encyclopedia of Genes and Genomes) and GO (Gene Ontology)
KEGG and GO databases and tools are used to analyze gene functions and interactions in the proposed research study. The signaling pathways such as PI3K-Akt, AGE-RAGE, HIF-1, TNF, are involved in the study to understand the critical aspects of cellular function, such as inflammation, metabolism, and cell survival. This study is examining how the formula affects these crucial pathways, which could be linked to its therapeutic effects on uterine adhesions.

Pathways included
The pathways mentioned (PI3K-Akt, AGE-RAGE, HIF-1, TNF, and IL-17) are specific types of signaling pathways involved in critical aspects of cellular function, such as inflammation, metabolism and cell survival. This suggests the study is examining how the formula affects these crucial pathways, which could be linked to its therapeutic effects on uterine adhesions.

Autodock Vina
This is a software tool used for docking simulations, which helps predict how small molecules, such as drugs, bind to a receptor of a protein. Strong binding between molecules typically suggests a higher potential for therapeutic effects.

Therefore, putting it all together, the study uses advanced databases and tools to analyze how specific components of the "Zishen Huayu Fang" formula might interact with molecular pathways and proteins that are implicated in the formation and treatment of intrauterine adhesions. The aim is to scientifically validate the traditional uses of this

formula and understand its potential mechanisms of action at a molecular level. By demonstrating strong binding between certain plant-derived compounds in the formula and key proteins involved in these pathways, the study suggests that these interactions might underlie the therapeutic effects of the formula.

RESULTS

Active targets and ingredients of "Zishen Huayu Fang"
Active ingredients and targets of "Zishen Huayu Fang" are extracted through TCMSP, Batman TCM and HERB databases. There are altogether 131 active drugs and 440 targets of Zishen Huoxue formula obtained after summarization.

Uterine adhesions disease targets and Venn maps
GeneCards, NCBI and DisGeNET databases are used to retrieve target genes related to uterine adhesions. 3644 targets have been taken from the GeneCards database; an additional 115 targets have been extracted from the NCBI database; 24 targets have been acquired from the DisGeNET database. 3649 target genes related to intrauterine adhesions are obtained after merging and pruning. By Venny 2.1.0 online mapping software, we have obtained the Venn map of the active ingredient-related targets and uterine adhesion-related targets of the "Zishen Huayu Fang", and also obtained 256 drug disease intersection targets.

PPI network formation in treating intrauterine adhesions by "Zishen Huayu Fang"
The common targets of were entered into the String database ANMELY "Zishen Huayu Fang"-intrauterine adhesions. The PPI network diagram was drawn through Cytoscape 3.8.0 software (fig. 1). It comprises 256 nodes and 1065 edges, with 8.32 as average degree value. The color turns redder when the node becomes larger, indicating high relativity. The thicker line indicates more important connections.

Topology analysis and MCODE clustering analysis
Using the Network Analyzer tool, we have conducted topological analysis on the PPI network after importing it into Cytoscape 3.8.0 (fig. 2). Using degree sorting, 85 important targets have been chosen based on genes with degree values that were higher than average. The top 20 targets included RXRA, CASP8, FOS, P4HA1, P4HA2, EGFR, VEGRA, IL10, MYC, STAT3, MAPK1, AKT1, HSP90AA1, JUN, CTNNB1, RELA, ITGB3, ESR1, IL6, MAPK14, and RXRA. The degree value of each target is used as the horizontal coordinate when images are plotted using R 4.0.3. After performing core target screening and MCODE gene cluster analysis, 14 gene clusters and 11 core genes are formed including RB1, INSR, COL3A1, PCNA, PRKCA, COL9A2, POR, EGF, IGFBP3, MMP1, PYCR1 (fig. 3).

Table 1: Key ingredients of "Zishen Huayu Fang" for the treatment of uterine cavity adhesions

Serial number	MOLID	English name	Chinese name	Degree value
1	MOL000098	Quercetin	槲皮素	122
2	MOL000006	Luteolin	木犀草素	51
3	MOL000422	Kaempferol	山萘酚	48
4	HBIN029753	Hydroxyproline	羟脯氨酸	45
5	MOL013179	Fisetin	漆黄素	40
6	MOL000358	Beta-sitosterol	β-谷甾醇	36
7	MOL005828	Nobiletin	川陈皮素	30
8	MOL001439	Arachidonic acid	花生四烯酸	29
9	MOL000354	Isorhamnetin	异鼠李素	27
10	MOL007154	Tanshinone II a	丹参酮 II a	26

Table 2: The binding energies of eight key targets corresponding to their interacting compounds

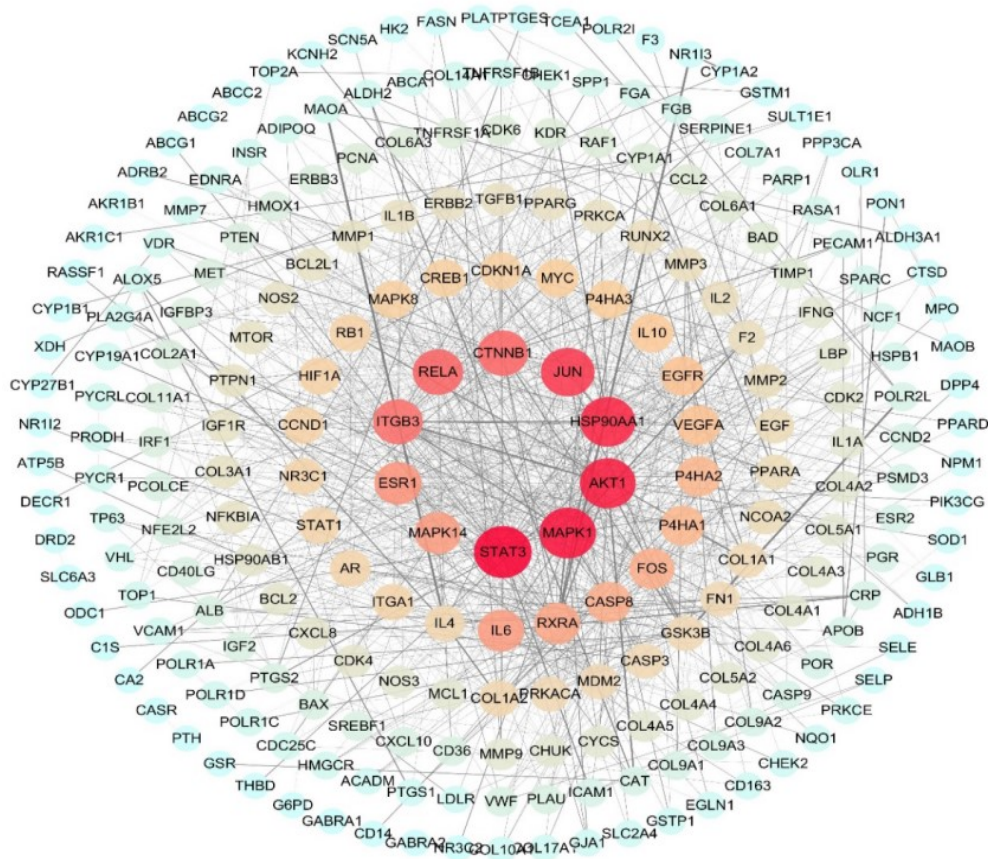| Targets	PDB ID	Compounds	Combined energy (kcal/mol^{-1})
AKT1	2UVM	kaempferol	-6.2
		quercetin	-6.2
MAPK1	2Y9Q	kaempferol	-7.6
		quercetin	-7.3
STAT3	6NJS	Kaempferol	-7.9
		Quercetin	-7.0
HSP90AA1	1OSF	Luteolin	-8.0
JUN	5T01	Luteolin	-6.0

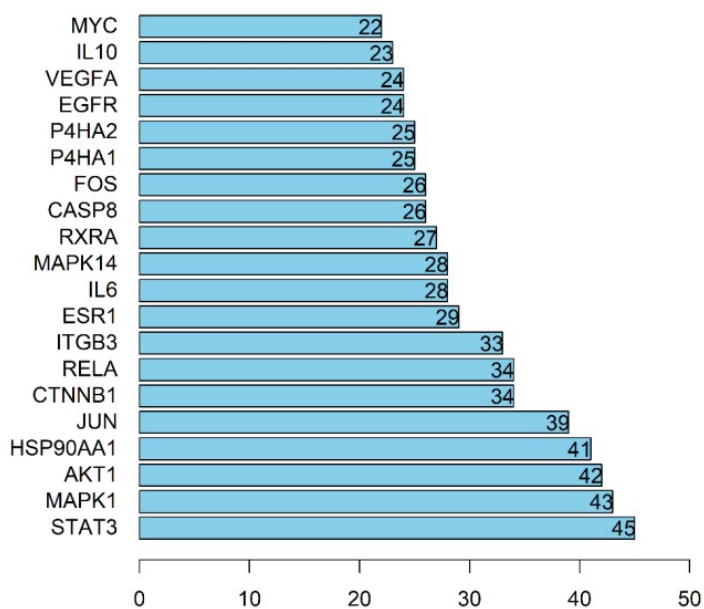**Fig. 1**: PPI network of target points for treating uterine adhesions with "Zishen Huayu Fang"

Fig. 2: Topology analysis of key targets in PPI network

Network diagram of drug-active ingredient-uterine adhesion-target and of key ingredient screening in "Zishen Huayu Fang"

Based on the incorporated active ingredients of "Zishen Huayu Fang", uterine adhesions and their targets, a network diagram of active ingredient-uterine adhesions-target of "Zishen Huayu Fang" has been drawn by Cytoscape 3.8.0 (fig. 4). Topology analysis is conducted to obtain the key active ingredients of "Zishen Huayu Fang" for the treatment of uterine adhesions. The top ten key ingredients are quercetin, lignocaine, sannaphene, hydroxyproline, laccasein, β-sitosterol, Chuan Chen Pi, arachidonic acid, isorhamnetin and tanshinone IIa in order of degree value (table 1).

Key targets GO enrichment analysis of "Zishen Huayu Fang" for Uterine Adhesions

This study has thoroughly interpreted GO and KEGG enrichment analysis results as it is very important and crucial to determine the interconnections among biological processes, molecular functions, cellular components and signaling pathways. We have initially identified the overlapping biological processes and then we have examined their hierarchical relationships to infer the broader implications. Our study has also investigated how enriched cellular components interact and how do they play their respective roles in performing specific functions. The study also considers the functional synergy of molecular functions within appropriate pathways.

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2491 biological processes (BP) are augmented in the GO enrichment analysis, primarily involving oxidative stress response, nutrient level regulation and other processes; 85 cellular components (CC) are supplemented mainly involving collagen, extracellular matrix and other parts of the treatment. 218 molecular functions (MF) have been enriched involving extracellular matrix structural components, extracellular matrix structural components, and extracellular matrix structural components. 218 enriched molecular functions (MF) mainly involve extracellular matrix structural components and DNA binding transcription factors.

Enrichment analysis of KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway, a key target of "Zishen Huayu Fang" for the treatment of uterine adhesions

The KEGG analysis provides the information about the biological pathways linked with the databse by determining the significantly enriched pathways important for protein abundance or gene expression. Each pathway shows a series of molecular interactions along with the biological processes, which eventually helps in analysing the enrichment scores such as adjusted p-values linked to each pathway.
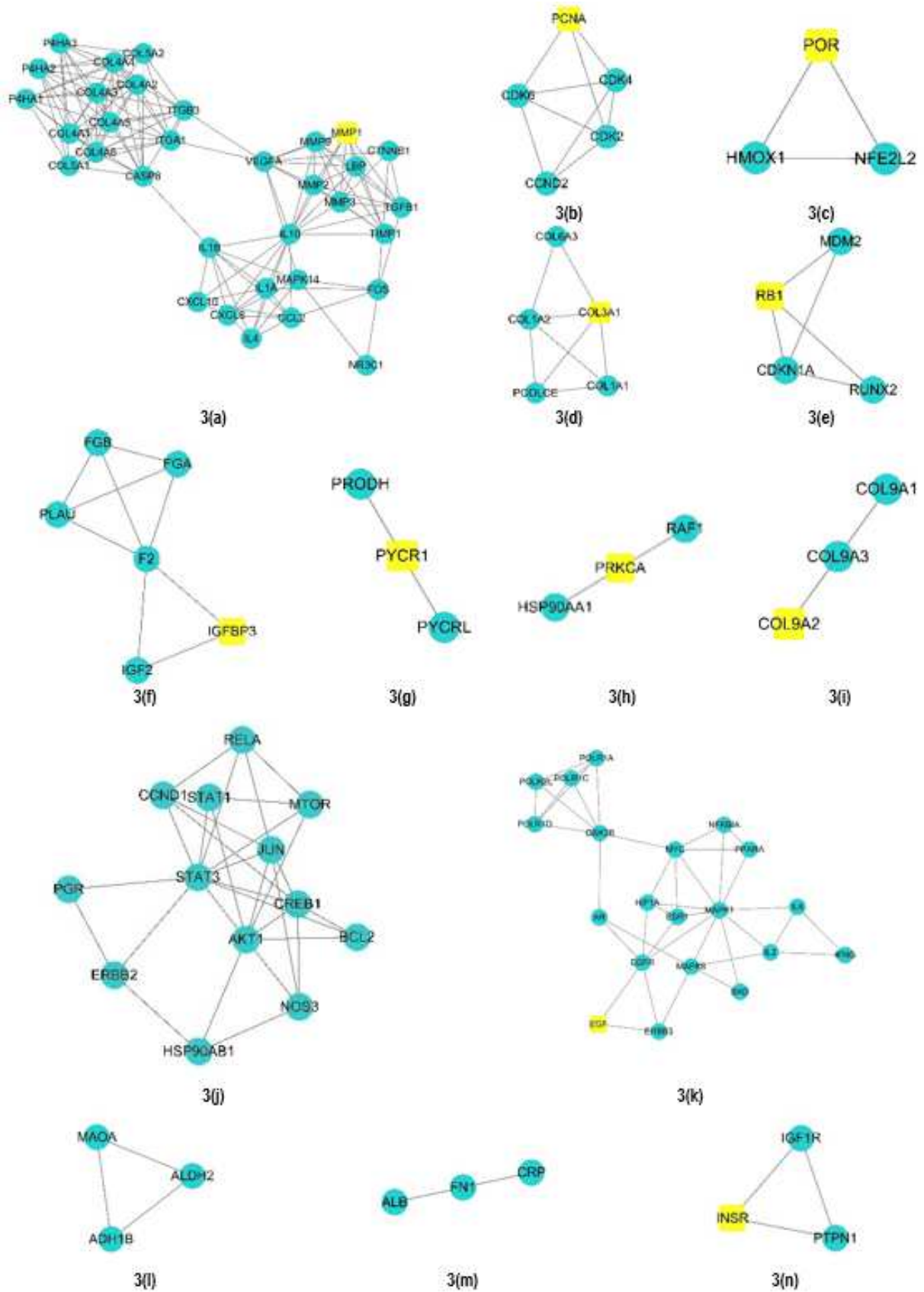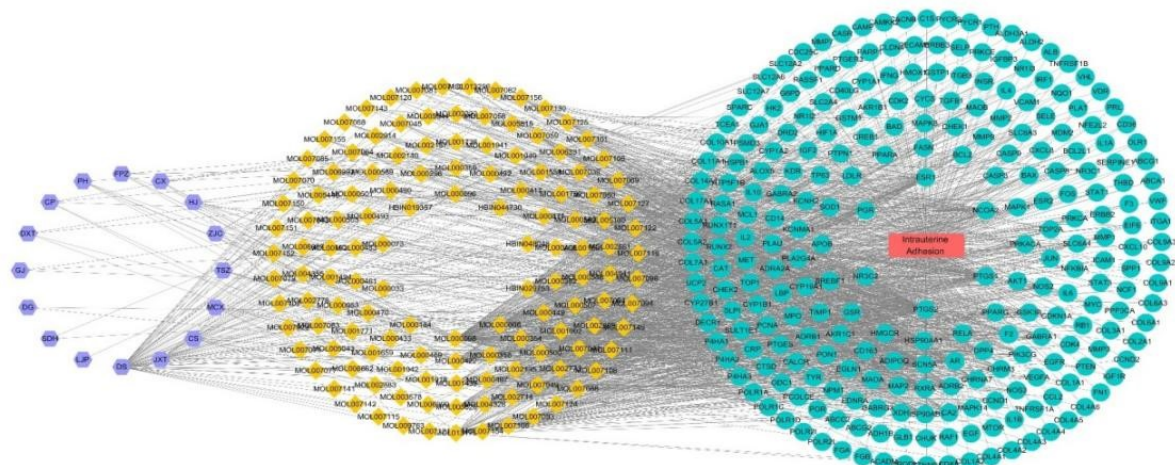
Fig. 3: Gene clusters of MCODE

Fig. 4: Network diagram of drug-active ingredient-uterine adhesion-target in "Zishen Huayu Fang"
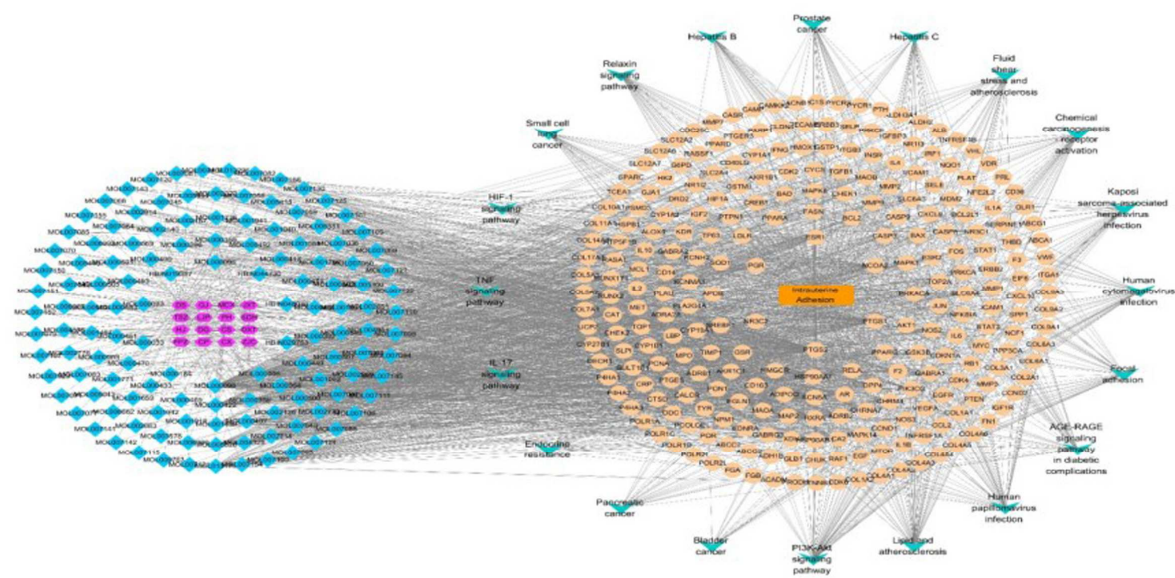

Fig. 5: Drug-active ingredient-uterine adhesions-pathway-target network of "Zishen Huayu Fang"

More important pathways designate a strong association with our genes of interest and can show the significant biological mechanisms. Identifying common proteins or genes that link multiple pathways elucidate the regulatory networks. KEGG pathway can help to determine whether the genes of the patient fit within these pathways, by demonstrating their interactions and respective roles.

It is indicated from the findings after enrichment analysis of KEGG pathway that altogether 172 pathways of signaling are enriched, mainly involving PI3K-Akt, AGE-RAGE, HIF-1, TNF and IL-17.

Network formation of drug-active ingredient-uterine adhesion-pathway-target of "Zishen Huayu Fang"
"Zishen Huayu Fang" maintains the synergistic features of many targets, multiple components and several pathways in healing uterine adhesion. The target network

and enrichment analysis in drug-active ingredient-uterine adhesion of "Zishen Huayu Fang" have been integrated to draw a network chart by Cytoscape 3.8.0 in order to give a visual display (fig. 5).

Docking analysis of molecular key target and active ingredients proteins in "Zishen Huayu Fang"
In order to conduct a decomposition analysis for identifying the energy contributions from specific residues, molecular docking provides information on key interactions and helps to determine the critical factors for binding. This involves key interactions between the proteins such as determination of hydrogen bonds and hydrophobic contacts. If the results generate multiple poses then cluster analysis is peformed as done in our study to reveal the most representative binding conformations.
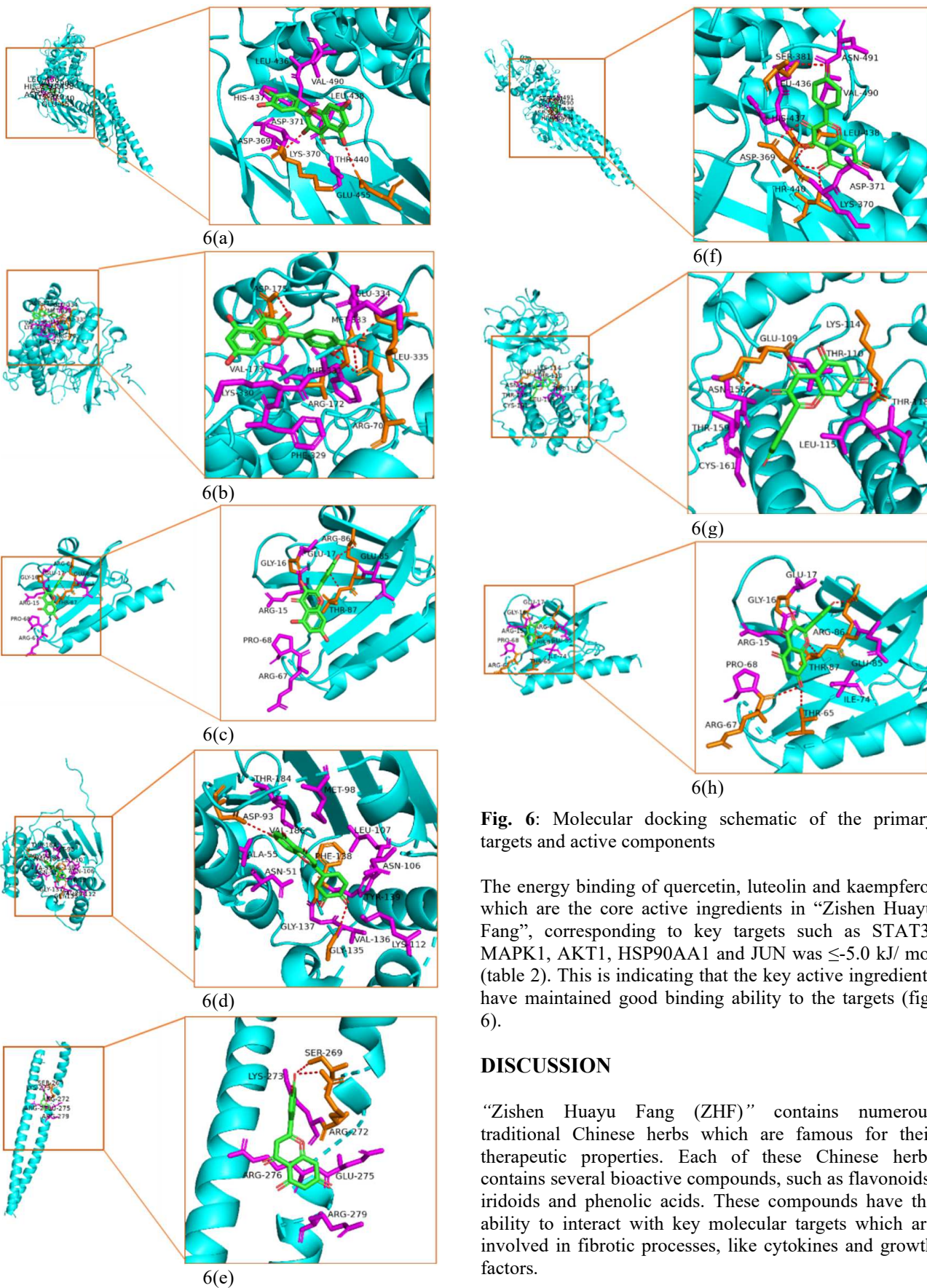

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6(a)

6(b)

6(c)

6(d)

6(e)

6(f)

6(g)

6(h)

Fig. 6: Molecular docking schematic of the primary targets and active components

The energy binding of quercetin, luteolin and kaempferol which are the core active ingredients in "Zishen Huayu Fang", corresponding to key targets such as STAT3, MAPK1, AKT1, HSP90AA1 and JUN was ≤-5.0 kJ/ mol (table 2). This is indicating that the key active ingredients have maintained good binding ability to the targets (fig. 6).

DISCUSSION

"Zishen Huayu Fang (ZHF)" contains numerous traditional Chinese herbs which are famous for their therapeutic properties. Each of these Chinese herbs contains several bioactive compounds, such as flavonoids, iridoids and phenolic acids. These compounds have the ability to interact with key molecular targets which are involved in fibrotic processes, like cytokines and growth factors.

The connection between molecular targets and their related signaling pathways is essential to understand the therapeutic effects of ZHF. The TGF-β pathway regulates fibrosis and inflammation. With the aid of TGF-β signaling, ZHF decreased collagen synthesis and then promote tissue repair for mitigating intrauterine adhesions. The network pharmacology also enhances the treatment given by ZHF as it helps in mapping the interactions between multiple targets, active components and pathways.

The active components of ZHF are able to bind to specific enzymes and receptors influencing cellular behaviors that contribute to intrauterine adhesion formation. For example, flavonoids from Angelica sinensis may obstruct the production of fibroblasts and decrease collagen deposition by controlling the growth factor-beta (TGF-β). These binding are analysed through molecular docking which helps ZHF to prevent excessive scar tissue formation in the uterus.

The modern medical theories helps in understanding the role of "Zishen Huayu Fang" (ZHF) in treating intrauterine adhesions by considering the factors like antioxidant, anti-inflammatory and anti-fibrotic effects. The ZHF's active components, such Rehmannia glutinosa and Angelica sinensis, show relevant antioxidant properties. ZHF controls inflammatory pathways by hindering pro-inflammatory cytokines like IL-6 and TNF-α, and creates more conducive environment for healing. Its anti-fibrotic action is significant as it constrains the TGF-β pathway, which is very important in scar formation. These mechanisms in integration create an effect that stimulates normal uterine healing. Through network pharmacology, it is found from this study found that Quercetin, Luteolin, kaempferol, etc. are the main active ingredients of ZHF in treating uterine cavity adhesion. Modern pharmacological research shows that Quercetin owns strong biological activity, which can play an antioxidant part by reducing oxidative stress, down-regulation of signaling route downstream that is controlled by reactive oxygen species (Wang *et al*., 2023). It can reduce the expression of inflammatory factors such as interleukin-1 (IL-1), IL-6 and IL-10 (Lu *et al*., 2023).

It can play an antifibrotic role by up-regulating miR-145 and inhibiting the activation of TGF-β1/Smad2/Smad3 pathway (Xu *et al*., 2020). Thus it was suggested that the key active ingredients of "Zishen Huayu Fang" may mainly inhibit the pathological process of uterine adhesions and prevent them by regulating oxidative stress, anti-inflammatory, and anti-fibrosis processes (Zhao *et al*., 2023).

Topology analysis of key targets revealed that gene targets such as STAT3, MAPK1, AKT1, HSP90AA1, JUN, etc. may be active in treating uterine adhesions with ZHF. Currently, plenty of studies have confirmed that inhibiting the secretion of STAT3, MAPK1 and AKT1 proteins can effectively alleviate the inflammatory response during endometrial repair and reduce the degree of uterine adhesions (Shi *et al*., 2023); HSP90AA1 and JUN have strong immune response effects, and playing important regulatory roles in inflammatory response, clearing free radicals, as well as improving antioxidant enzyme activity The results of molecular docking in this study showed that quercetin and Kaempferol had good binding properties with STAT3, MAPK1 and AKT1 and luteolin had good binding properties with HSP90AA1 and JUN. The results showed that Quercetin, Kaempferol and Luteolin may be the core active ingredients of "Zishen Huayu Fang" in treating IUA.

It can also be inferred through GO enrichment analysis that the "Zishen Huayu Fang" may reduce collagen fiber secretion and extracellular matrix deposition, prevent and treat uterine adhesions, and reduce the degree of adhesions by regulating biological processes such as oxidative stress and nutritional levels in patients with uterine adhesions. The results of KEGG pathway enrichment analysis mainly involve inflammatory reactions, viral infections and other aspects (Yang *et al*., 2021).

It has been determined through researches that signal pathways such PI3K-Akt, AGE-RAGE, HIF-1, TNF and IL-17 are intimately related to the pathological development of uterine adhesions. It is speculated that these pathways may be the key signaling pathways in the treatment of IUA with Zishen Huayu formula (Zhang *et al*., 2020). Oxidative stress can accelerate the formation of AGEs, activate the AGE-RAGE pathway, regulate the oxidase system in epithelial cells, promote the expression of inflammatory factors and lead to cell inflammatory damage and apoptosis (Wu *et al*., 2021). TNF and IL-17, as inflammatory mediators and immune regulators, can induce fibroblasts, vascular endothelial cells, etc. to release macrophage Colony-stimulating factor and regulate T helper cell 17 (Th17) to cause tissue inflammatory damage (Zhao *et al*., 2022; Zhong *et al*., 2022).

Activation of the above four pathways can cause inflammatory response to stimulate vascular contraction and aggravate endometrial ischemia and hypoxia. When the injured endometrium is hypoxic, hypoxia inducible factor-1 (HIF-1) induces p53 to up-regulate the cell cycle process, resulting in the accumulation of G2/M cells and activates TGF-β, CTGF and other factors to regulate its downstream target genes such as STAT3 to regulate the body's angiogenesis and promote the occurrence of fibrosis (Zhang *et al*., 2022). The mass secretion of AGEs can also stimulate mononuclear macrophages to secrete TNF-α, IL-1 and other factors to promote the expression of connection in the middle layer of the cell matrix,

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induce the increase of matrix synthesis in mesothelial cells, resulting in the accumulation of a large number of matrix proteins or the structural changes of matrix components modified by AGEs and eventually induce pathological changes of fibrosis (Zhang Tian *et al*., 2022).

In summary, "Zishen Huayu Fang" may regulate key targets including STAT3, MAPK1, AKT1, HSP90AA1, JUN through Quercetin, Luteolin, kaempferol and other active ingredients that regulate PI3K-Akt, AGE-RAGE, HIF-1, TNF, IL-17 and other related signal pathways, inhibit oxidative stress and inflammatory response. It can also reduce endometrial fibrosis and effectively prevent and treat uterine adhesion. This study preliminarily explored the basic pharmacological effects and related mechanisms of ZHF through network pharmacology, providing certain reference and guidance for clinical application.

CONCLUSION

Zishen Huayu Fang is a traditional Chinese herbal formula used for various gynecological conditions. It has been specifically studied for its potential benefits in treating uterine adhesions, which are bands of fibrous scar tissue that form inside the uterus and can lead to infertility, menstrual pain and abnormal menstrual bleeding. In conclusion, "Zishen Huayu Fang" exhibits potential therapeutic effects on uterine adhesions by targeting key genes such as STAT3, MAPK1, AKT1, HSP90AA1 and JUN, as identified through network pharmacology. The active ingredients, including Quercetin, Luteolin and kaempferol, contribute to the regulation of PI3K-Akt, AGE-RAGE, HIF-1, TNF and IL-17 pathways, ultimately inhibiting oxidative stress and inflammatory responses. This multi-target, multi-pathway approach suggests "Zishen Huayu Fang" as a promising intervention to prevent and treat uterine adhesions, providing valuable insights for future clinical applications.

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