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Mechanistic investigation of Kuntai capsule in endometriosis treatment: A network pharmacology, molecular docking, and molecular dynamics simulation approach

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ABSTRACT

Objective: To explore the mechanism of action of Kuntai capsule in the treatment of endometriosis (EMT).
Methods: The active components and corresponding targets of Kuntai capsule were obtained from the TCMSP, BATMAN-TCM, Pubchem, and SwissTargetPrediction databases. EMT-related disease targets were retrieved from GeneCards, DisGeNET, TTD, OMIM, and Drugbank. A Venn diagram was employed to identify the intersection targets of Kuntai capsule and EMT. The disease-component-target network was constructed using Cytoscape, and the common target protein-protein interaction (PPI) network was built using the STRING database. Topological analysis of the PPI network was performed using Cytoscape to screen for core targets. Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were performed using the DAVID database. Molecular docking was performed with AutoDockTools. The stability of the optimal binding energy model was further validated using GROMACS molecular dynamics simulations.
Results: A total of 182 common targets were identified. The core components included sitosterol, panicolin, and rivularin. Among them, TNF, GAPDH, and AKT1 were found to play significant roles in the biological network of Kuntai capsule in treating EMT. These core targets are primarily involved in processes such as the negative regulation of apoptosis pathway and oncogenic pathway such as the PI3K-Akt signaling, which plays a therapeutic role in EMT. Molecular docking and molecular dynamics simulations further confirmed the stable and tight binding of sitosterol to AKT1.
Conclusion: Kuntai capsule may exert therapeutic effects in EMT by activating multiple signaling pathways through the regulation of core targets such as TNF. These findings not only enhance our understanding of the mechanism of action of Kuntai capsule but also provides new insights into the potential clinical applications of traditional Chinese medicine (TCM) in EMT treatment. Future research can further explore how TCM drugs can intervene in the pathological processes of EMT.

Introduction

Endometriosis (EMT) is an estrogen-dependent chronic inflammatory condition characterized by the growth of endometrial-like tissue outside the uterine cavity. The pathogenesis of EMT is related to factors such as immune dysfunction, sex hormones, oxidative stress, and genetic predisposition.[1,2] Clinical manifestations of EMT often include chronic pelvic pain, dysmenorrhea, and infertility.[3] It occurs in about 10 % of women of reproductive age and 50 % of women with fertility-related issues.[4] While current Western medical treatments, including surgery, non-steroidal anti-inflammatory drugs (NSAIDs), and hormone therapy, can alleviate certain symptoms of EMT, these therapies are often accompanied by adverse effects and a high rate of recurrence.[5] Herbal compounded oral medicines are increasingly used as alternative therapies for EMT, due to their advantages such as multi-targeted action and relatively lower incidence of side effects.[6]

In traditional Chinese medicine, blood stagnation is considered the fundamental pathological factor and a central element in the development of endometriosis.[6] Kuntai capsule, derived from Huanglian Ejiao Decoction in Zhang Zhongjing's *Treatise on Miscellaneous Diseases of*

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Typhoid Fever, is known for its effectiveness in calming the mind, alleviating vexation, nourishing yin, and clearing heat. Studies suggest that Kuntai capsule exhibits phytoestrogen-like effects, which can enhance blood flow to the endometrium, promote endometrial thickening, and improve endometrial receptivity.[7] Furthermore, it is reported to increase the ovulation rate and improve pregnancy outcomes.[8] Several studies have indicated that Kuntai capsule effectively alleviates perimenopausal symptoms induced by gonadotropin-releasing hormone agonist (GnRH-a) treatment.[9,10] However, the main pharmacological mechanisms and underlying molecular mechanisms of Kuntai capsule in treating endometriosis remain insufficiently explored. This study aims to utilize network pharmacology, molecular docking, and molecular dynamics simulations to explore the therapeutic mechanisms of Kuntai capsule for EMT by identifying the core components, key targets, and potential pathways.

Materials and methods

Collection of active ingredients in Kuntai capsule

Kuntai capsule is a compound herbal formula comprising 6 herbs: Radix Rehmanniae Praeparata (shudihuang), Rhizoma Coptidis(huanglian), Radix Paeoniae Alba (baishao), Radix Scutellaria Baicalensis (huangqin), Poria Cocos (fuling), and Donkey hide gellatin (ejiao). Active ingredient data for Radix Rehmanniae Praeparata, Rhizoma Coptidis, Radix Paeoniae Alba, Radix Scutellariae Baicalensis, and Poria Cocos were retrieved from the Traditional Chinese Medicine Systems Pharmacology Platform (TCMSP, http://tcmspw.com/index.php).[11] Donkey Hide Gelatin could not be found in the TCMSP database, so its active ingredients were sourced from the A Bioinformatics Analysis Tool of Molecular Mechanism of Traditional Chinese Medicine (BATMAN-TCM, http://bionet.ncpsb.org.cn).[12]

Screening of active ingredients and targets of Kuntai capsule

Active ingredients of Kuntai capsule were screened using the TCMSP and BATMAN-TCM platforms. Criteria for selection in TCMSP included an oral bioavailability (OB) $\geq$ 30 % and drug-likeness (DL) $\geq$ 0.18.[13] The corresponding active ingredients and their targets were identified as SMILE through the PubChem database (https://pubchem.ncbi.nlm.nih.gov) and were imported into the SwissTargetPrediction database (http://www.swissadme.ch),[14] with a probability $>$ 0 set as the screening criterion. Redundant data were removed after pooling the results. For Donkey Hide Gelatin, data were retrieved from the BATMAN-TCM database, with a score cutoff of 30 and a P-value threshold of 0.05 to obtain its relevant targets.

Acquisition of EMT-related targets

The keyword “endometriosis” was used to search in five major disease gene databases: GeneCards (https://www.genecards.org), DisGeNET (https://www.disgenet.org), OMIM (http://www.omim.org), DrugBank (https://www.drugbank.ca), and TTD (https://db.idrblab.net/ttd). In the GeneCards and DisGeNET databases, a higher score indicates a stronger association with the disease. Targets with a score greater than the median were selected as potential targets for EMT. Since OMIM, DrugBank, and TTD databases do not provide a score, no screening was conducted in these cases. After combining the targets from all five databases, duplicate entries were removed to generate a final list of EMT-related targets.

Target screening at the intersection of drugs and diseases

The obtained active ingredient targets and disease targets were imported into the Venny 2.1.0 online software platform (http://bioinfogp.cnb.csic.es/tools/venny), and the software obtained a Venn diagram, which was used to screen the intersecting targets of Kuntai capsule for the treatment of EMT.

Construction of disease-component-target network

The disease-component-target network was constructed using Cytoscape software,[15] where nodes represent the factors (disease, components, and targets), and edges indicate their interrelationships. The degree value, calculated using the NetworkAnalysis plugin, reflects the number of connections for each node in the network. A higher degree indicates that the node is more pharmacologically active and likely to serve as a key core component in the treatment of EMT.

Table 1
Basic Information on Some Active Ingredients of Kuntai Capsule.

Source	MOL ID	Ingredient	OB	DL
Radix Paeonia Alba	MOL001918	paeoniflorgenone	87.59	0.37
Radix Paeonia Alba	MOL000359	sitosterol	36.91	0.75
Radix Scutellaria Baicalensis	MOL002932	panicolin	76.26	0.29
Radix Scutellaria Baicalensis	MOL000359	sitosterol	36.91	0.75
Rhizoma Coptidis	MOL000785	palmatine	64.6	0.65
Rhizoma Coptidis	MOL000098	quercetin	46.43	0.28
Poria Cocos	MOL000275	trametenolic acid	38.71	0.8
Poria Cocos	MOL000283	ergosterol peroxide	40.36	0.81
Radix Rehmanniae Praeparata	MOL000359	sitosterol	36.91	0.75
Radix Rehmanniae Praeparata	MOL000449	stigmasterol	43.83	0.76
Donkey hide gellatin	MOL000054	arginine	47.64	0.03
Donkey hide gellatin	MOL000055	lysine	29.33	0.02

Table 2
Repetitive Active Ingredients of Kuntai Capsule.

Source	MOL ID	Serial number
Radix Paeonia Alba、Radix Scutellaria Baicalensis	MOL000358	A
Radix Paeonia Alba、Radix Rehmanniae Praeparata、Radix Scutellaria Baicalensis	MOL000359	B
Radix Scutellaria Baicalensis、Radix Rehmanniae Praeparata	MOL000449	C
Rhizoma Coptidis、Radix Scutellaria Baicalensis	MOL001458	D1
Rhizoma Coptidis、Radix Scutellaria Baicalensis	MOL002897	D2

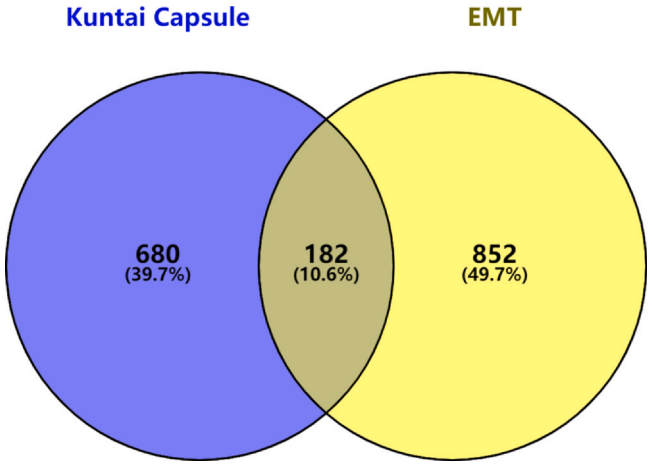

Fig. 1. Venn Diagram of Kuntai Capsule Targets and EMT Targets.

Construction of Protein-Protein Interaction (PPI) network and screening of core targets

The intersection targets under 1.4 were analysed by PPI through STRING database (https://string-db.org).[16] The species was set as "Homosapiens", the confidence interval was adjusted to equal to or $>$ 0.4, and the PPI results were imported into Cytoscap 3.10.1 software to construct the PPI network of potential targets for the treatment of EMT by Kuntai capsule. After topological analysis by Centiscape2.2 plug-in, the median of greater than degree value (degree), closeness centre value (closeness) and betweenness centre value (betweenness) was used as the threshold to screen the core network of Kuntai capsule for the treatment of EMT, and to screen the core targets.

Enrichment analysis of core targets of Kuntai capsule for the treatment of EMT

The core targets identified in section 1.6 were imported into the DAVID database (https://david.ncifcrf.gov) for Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. The GO enrichment analysis included three categories: Biological Process (BP), Cellular Component (CC), and Molecular Function (MF). The species was set as Homo sapiens, and a P-value $<$ 0.05 was used as the threshold for filtering.[17] The top 10 terms in BP, CC, and MF, as well as the top 20 pathways in KEGG, were selected and visualized. Additionally, the data were imported into a bioinformatics platform (www.bioinformatics.com.cn) to generate bar charts and bubble charts, which helped identify the primary biological functions and related signaling pathways.

Molecular docking validation

The top 5 core components (as identified in section 1.5) and the top 5 core targets (as identified in section 1.6) were subjected to molecular docking validation to assess the binding interactions between the targets and components, providing preliminary validation for the results of the network pharmacological analyses. The 3D structures of the core components in sdf format were obtained from the PubChem database. These sdf files were converted to pdb format using OpenBabel software. The protein structures of the core targets were retrieved from the PDB database (https://www.rcsb.org). Molecular docking was performed

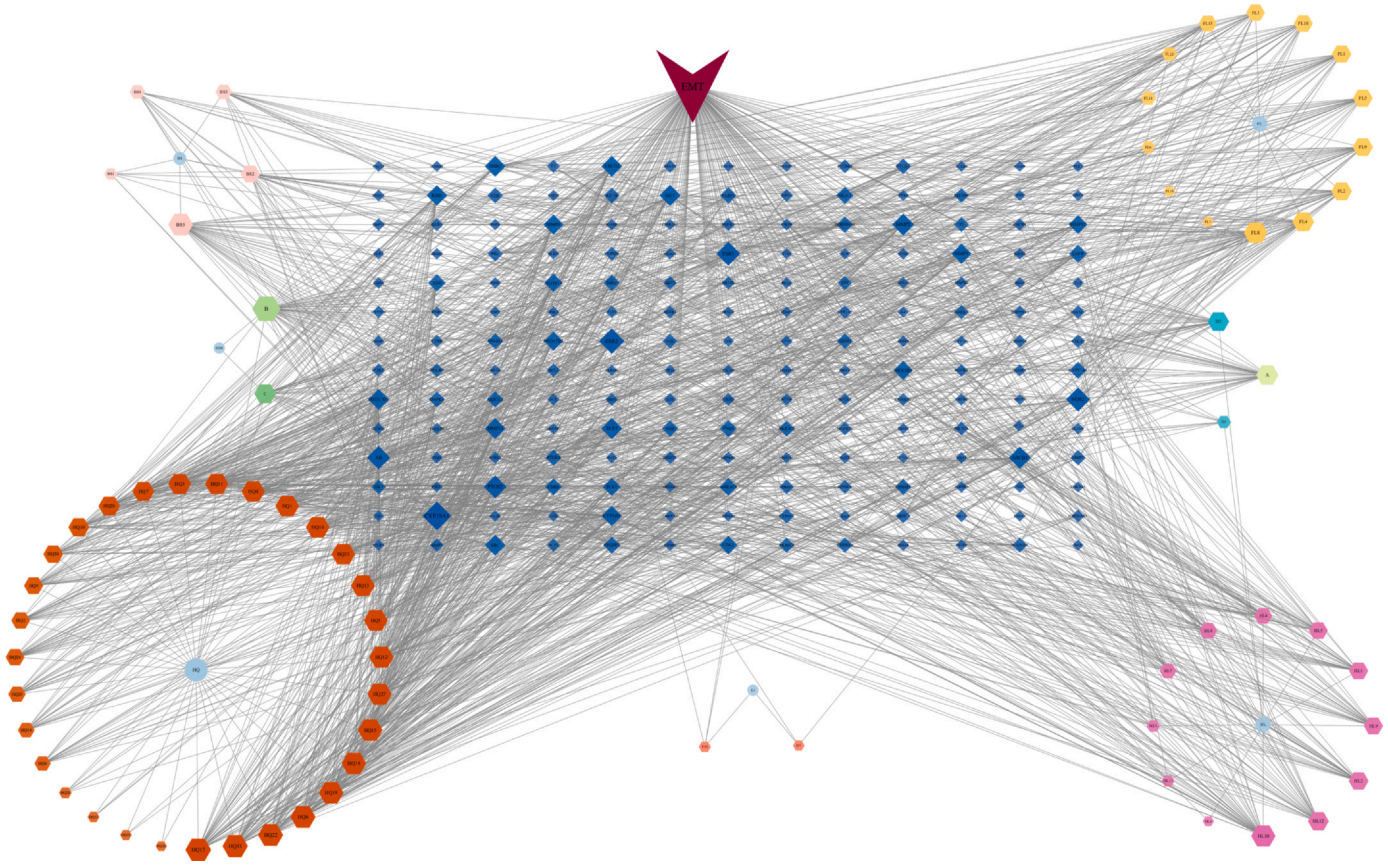

Fig. 2. The Potential Targets Network of active components. Note: Hexagonal nodes represent the active ingredients and rhombic nodes represent the active ingredient targets. The size of the area is proportional to the relative importance of both the active ingredients and their corresponding targets. HQ, Radix Scutellaria Baicalensis; HL, Rhizoma Coptidis; FL, Poria Cocos; BC, Radix Paeonia Alba; EJ, Donkey hide gellatin; SDH, Radix Rehmanniae Praeparata.

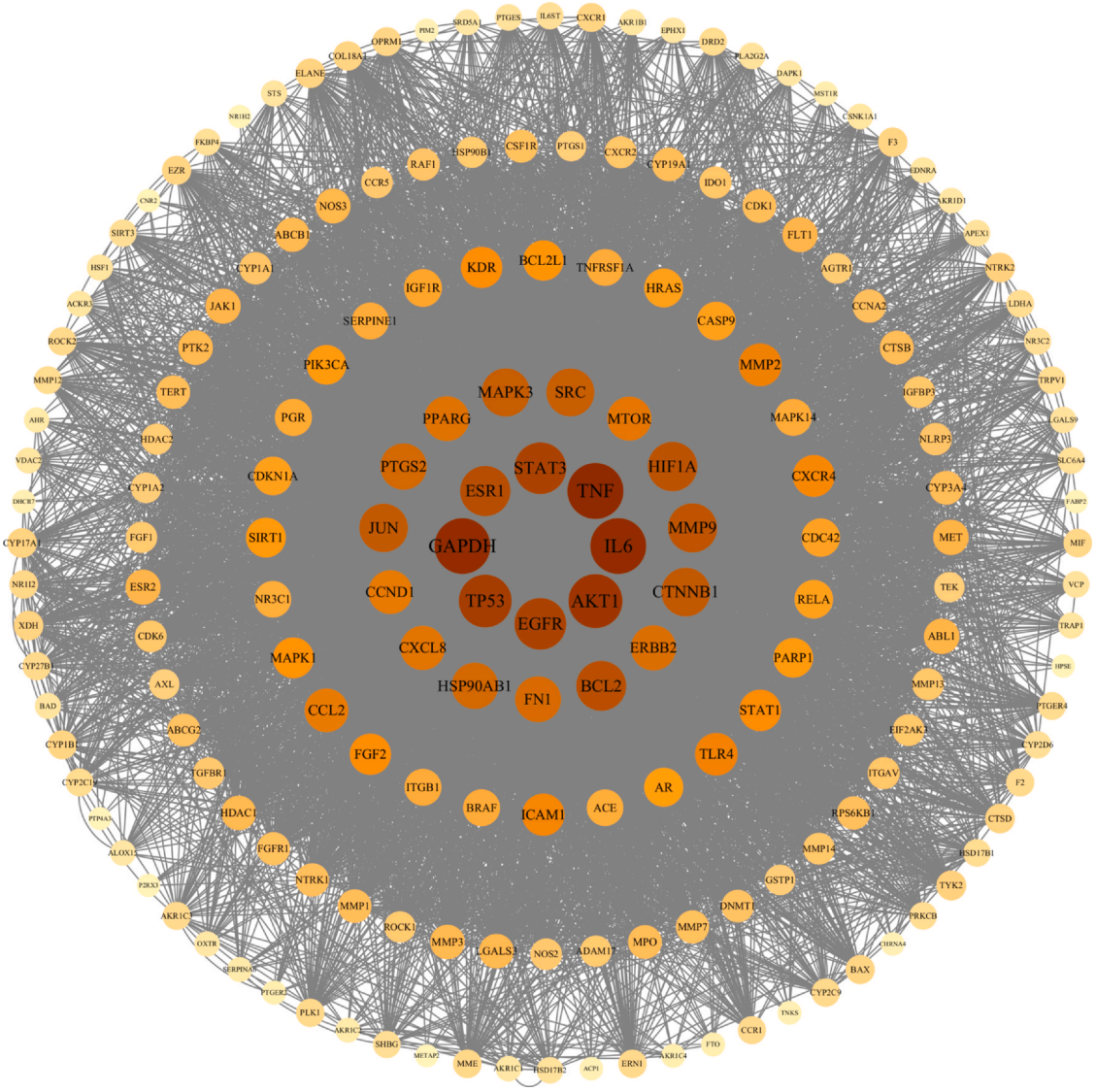

Fig. 3. PPI Network of Intersecting Targets. Note: The darker the colour and the larger the node, the higher the degree value of the target.

using AutoDockTools software.[18] Finally, the top 4 binding capacities were selected for visualisation using PyMOL software.

Molecular dynamics simulation

Molecular dynamics simulation captures the complex interactions and dynamics of molecules by modeling every atom and bond in the system. A molecular dynamics simulation with a total duration of 100 ns was performed using GROMACS [19] for the top 4 protein complexes with the strongest binding capacity (as identified in section 1.8). The molecular force field used for the kinetic simulations was Charmm36-mar2019 and the system was solubilized using the TIP3P water model. Energy minimization of the entire system was achieved through the steepest descent method. Prior to the production simulation, a 100 ps equilibration phase was conducted under a canonical ensemble (NVT) for constant volume and temperature, followed by a constant pressure-temperature (NPT) ensemble equilibration. During this phase, the temperature was maintained at 300 K, and the pressure was set to 1.0 bar. After equilibration, the production simulation was run with a time step of 2 fs, and the trajectory was saved every 100 ps. The simulation results were evaluated using Root Mean Square Displacement (RMSD), Radius of Gyration (RG), and Root Mean Square Fluctuation (RMSF) parameters.

Results and analysis

Prediction of active ingredients and potential targets of Kuntai capsule

The active ingredients of Kuntai capsule were retrieved from TCMSP and BATMAN-TCM databases. After screening and eliminating 6 untargeted ingredients, a total of 77 active ingredients were identified. These ingredients were derived from the following herbs: 36 from Radix Scutellaria Baicalensis, 14 from Rhizoma Coptidis, 14 from Poria Cocos, 8 from Radix Paeonia Alba, 3 from Donkey Hide Gelatin, and 2 from Radix Rehmanniae Praeparata. Using the SwissTargetPrediction tool, a total of 2687 active targets were obtained for Radix Scutellaria Baicalensis, 863 for Rhizoma Coptidis, 814 for Poria Cocos, 355 for Radix Paeonia Alba, 166 for Donkey Hide Gelatin, and 85 for Radix Rehmanniae Praeparata. After removing duplicate targets, 862 unique targets were identified for the active ingredients of Kuntai capsule. The basic information of some active ingredients and the number of duplicate chemical components are presented in Table 1 and Table 2, respectively.

Collection of EMT targets

A total of 1170 EMT targets were obtained from several disease databases, including GeneCards, DisGeNET, OMIM, DrugBank, and

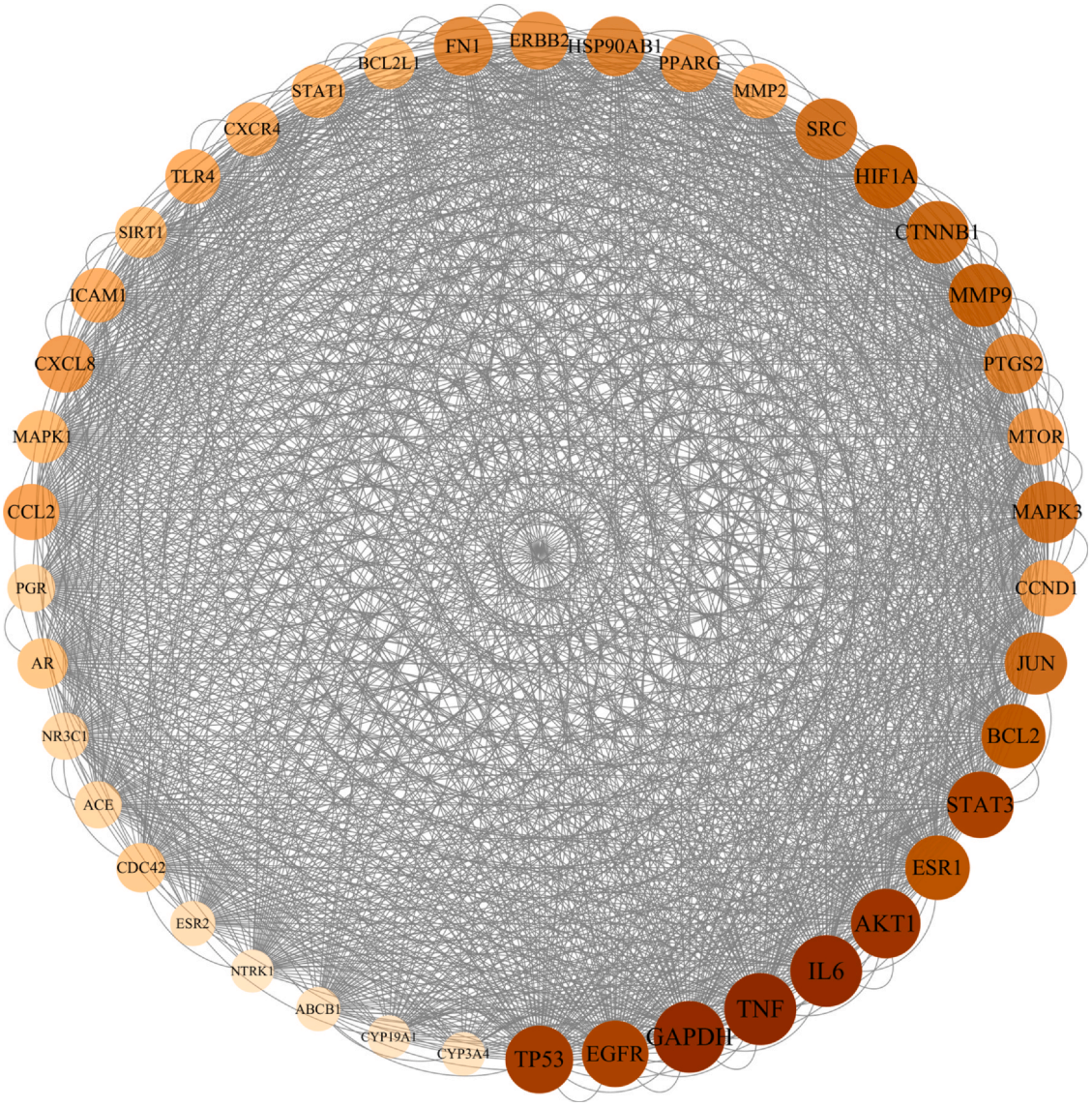

Fig. 4. PPI Network of Core Targets. Note: The darker the colour and the larger the node, the higher the degree value of the target.

TTD. After removing duplicate entries, 1034 unique EMT targets remained. The intersection of the drug targets and EMT targets revealed 182 intersecting targets, which are considered as potential therapeutic targets for Kuntai capsule in the treatment of EMT (Fig. 1).

Disease-component-target network of Kuntai capsule for EMT treatment

The 182 common targets of Kuntai capsule for the treatment of EMT were matched with the active ingredient-acting targets of Kuntai capsule, and a disease-component-target network was constructed (Fig. 2). The resulting network consisted of 258 nodes and 1929 edges. The NetworkAnalyzer plug-in function was used to analyze the topology of the network, with the size of the nodes being directly proportional to their degree index. This indicated that larger nodes had more connections, and thus were more critical targets in the network. The top 5 active ingredients were identified as sitosterol, panicolin, rivularin, 5,7,2,5-tetrahydroxy-8,6-dimethoxyflavone, and 5,2'-dihydroxy-6,7,8-trimethoxyflavone. These compounds were hypothesized to be the core components of Kuntai capsule for the treatment of EMT.

Construction of PPI network

The PPI network consists of 182 nodes and 7678 edges, with an average degree value of 42.2 (Fig. 3). To further explore the topological structure of the network, Centiscape plugin for Cytoscape was used for analysis. Based on the criteria established in section 1.6, specific thresholds for degree $\geq$ 84.373, closeness $\geq$ 0.003, and betweenness $\geq$ 157.615 were applied to identify the core targets involved in the treatment of EMT with Kuntai capsule. This resulted in the identification of 42 core targets. These core targets were then visualized and analyzed according to their degree size. The top five core targets identified were TNF (Tumor Necrosis Factor), GAPDH (Glyceraldehyde-3-Phosphate Dehydrogenase), IL6 (Interleukin 6), AKT1 (Serine/Threonine Protein Kinase), and TP53 (Tumor Protein P53) (Fig. 4). These targets are hypothesized to play significant roles in the biological processes associated with Kuntai capsule's therapeutic effects on EMT.

GO enrichment analysis

GO enrichment analyses were performed to characterize the specific targets of Kuntai capsule in treating EMT. A total of 773 entries were

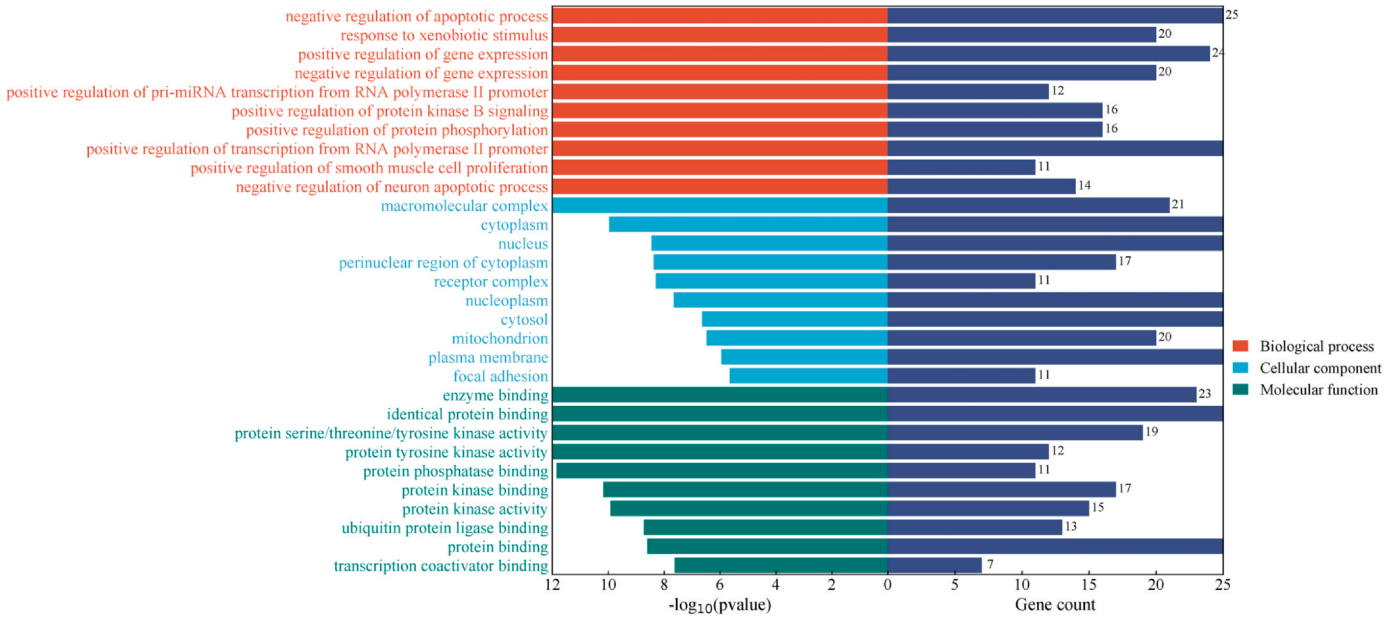

Fig. 5. GO Enrichment Analysis Bar Graph.

identified from the GO enrichment analysis, which included 607 BP terms, 65 CC terms, and 101 MF terms. The top 10 entries for BP, CC, and MF were selected by sorting the P-values (Fig. 5). The biological processes through which Kuntai capsule influences EMT primarily involve the negative regulation of apoptosis, the response to xenobiotic stimuli, and the positive and negative regulation of gene expression. Regarding cellular components, Kuntai capsule mainly targets the macromolecular complex, cytoplasm, nucleus, and perinuclear region of the cytoplasm. In terms of molecular function, the key processes are largely associated with enzyme binding, identical protein binding, and protein serine/threonine/tyrosine kinase activity.

KEGG pathway analysis

KEGG pathway analysis identified a total of 158 pathways. Using a bioinformatics platform, the top 20 pathways were visualized, and a bubble diagram of the KEGG pathway analysis was generated (Fig. 6). The KEGG pathway analysis revealed that the therapeutic effect of Kuntai capsule on EMT is closely associated with several key pathways, including those related to pathways in cancer, proteoglycans in cancer, PI3K-Akt signaling pathway, and lipid and atherosclerosis and other related signaling pathways.

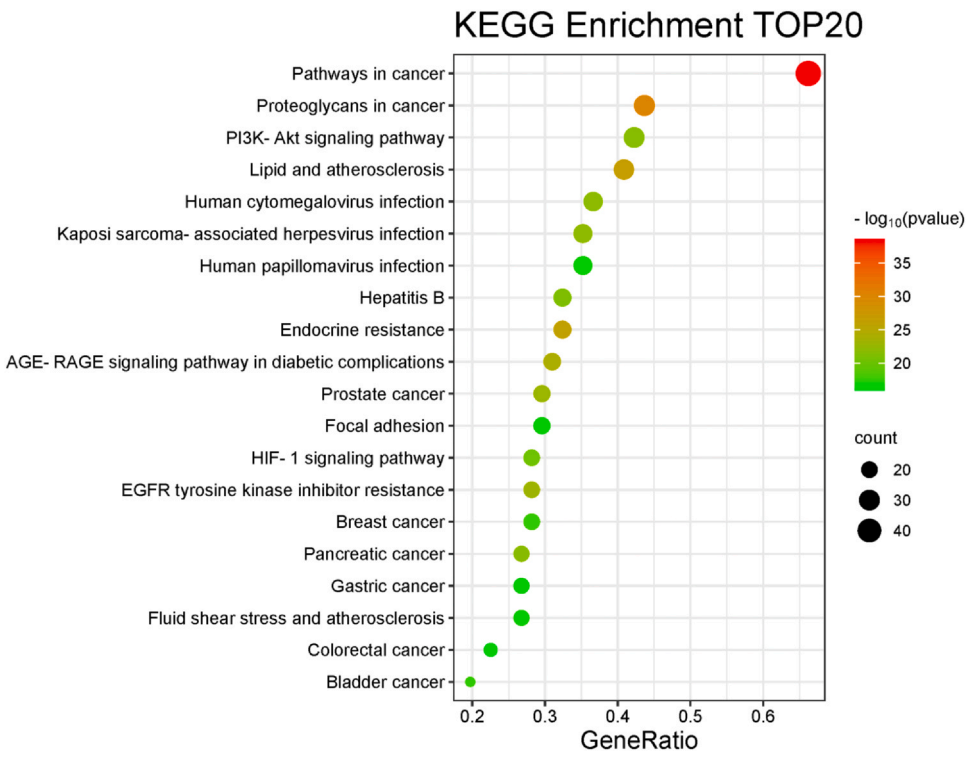

Fig. 6. KEGG Pathway Analysis Bubble Diagram.

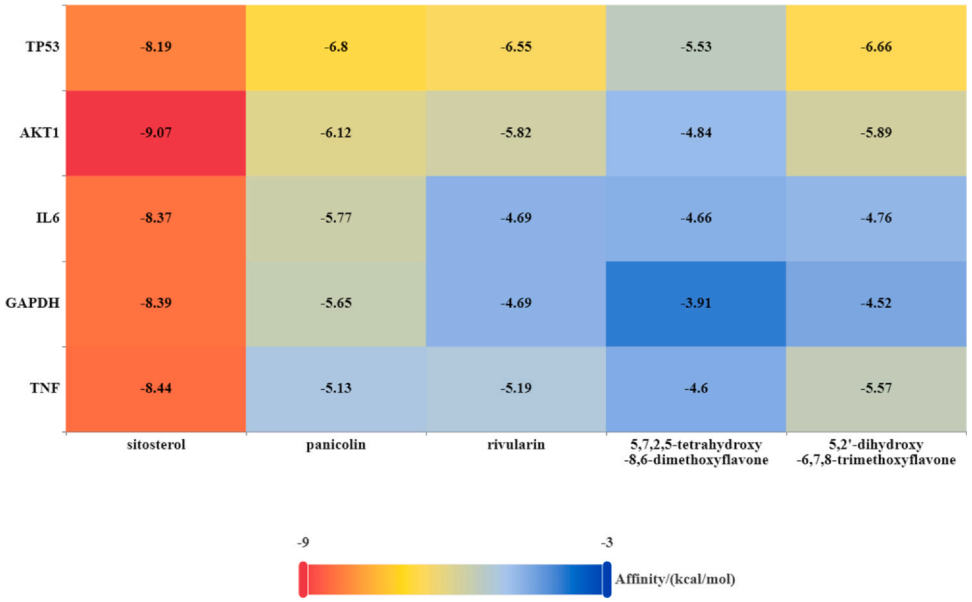

Fig. 7. Molecular Docking Heatmap.

Molecular docking validation

The top 5 core components identified in Section 2.3 were selected for molecular docking with the top 5 core targets identified in Section 2. The receptors used for docking included TNF (PDB ID: 7JRA), GAPDH (PDB ID: 4WNC), IL6 (PDB ID: 1ALU), AKT1 (PDB ID: 4GV1), and TP53 (PDB ID: 6GGC). According to the literature, receptor-ligand binding is considered more effective when the docking binding energy (Affinity) is ≤ -5.[20] The results of the lowest binding energies of receptor proteins and ligand small molecules are shown in Fig. 7. The docking patterns of the top 4 molecules with better binding are shown in Fig. 8.

Molecular dynamics simulation

Based on the molecular docking results in Section 2.7, sitosterol was selected for further molecular dynamics simulation to validate its interaction with IL6, AKT1, GAPDH, and TNF. Fig. 9 presents the RMSD of the proteins in the four target-ligand complexes during the kinetic simulation of 100 ns. RMSD is used to measure system stability. As shown in Fig. 9D, the RMSD of the AKT1 complex reached a more stable equilibrium with a very low level of fluctuation after an initial simulation of about 5 ns, indicating stable binding between the protein and ligand. The average RMSD of the AKT1 complex was 0.1879 nm, which was lower than that of the other three complexes (RMSD of 0.1972 nm for the IL6 complex), consistent with the molecular docking simulation results.

RMSF was used to describe the fluctuation of individual amino acid residues during the simulation process. Significant conformational changes or motions were observed in certain amino acid residues of the GAPDH (Fig. 10A) and TNF (Fig. 10C) complexes, with excessive fluctuations potentially affecting the stability of these complexes, as indicated in Fig. 9. In contrast, the RMSF of the IL6 (Fig. 10B) and AKT1

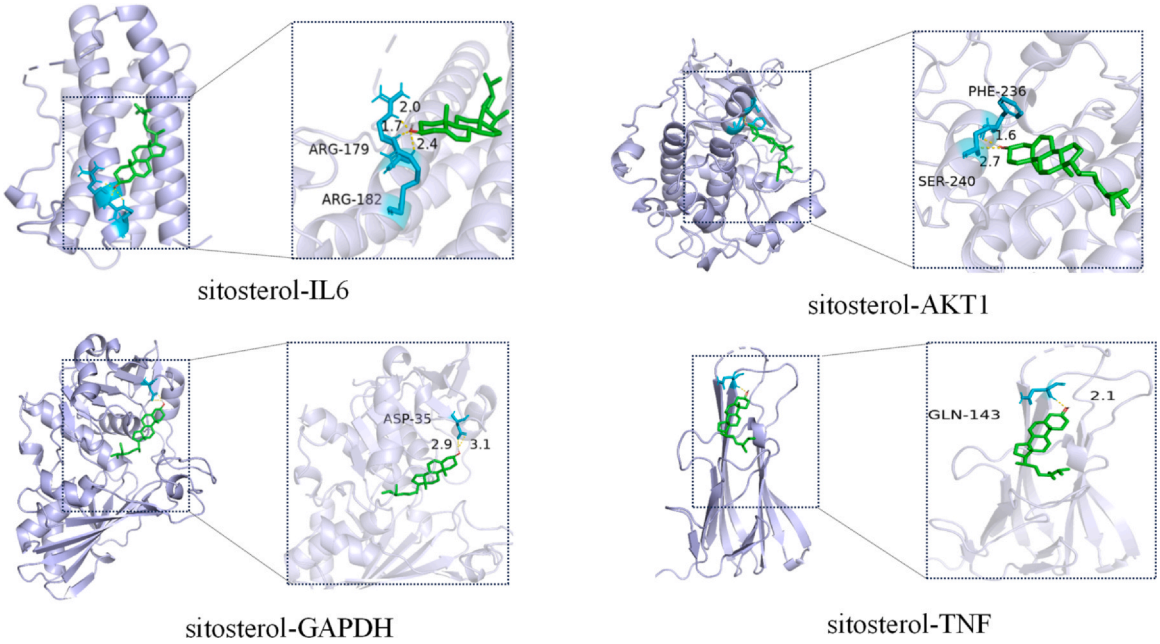

Fig. 8. Visualization Diagram of Molecular Docking.

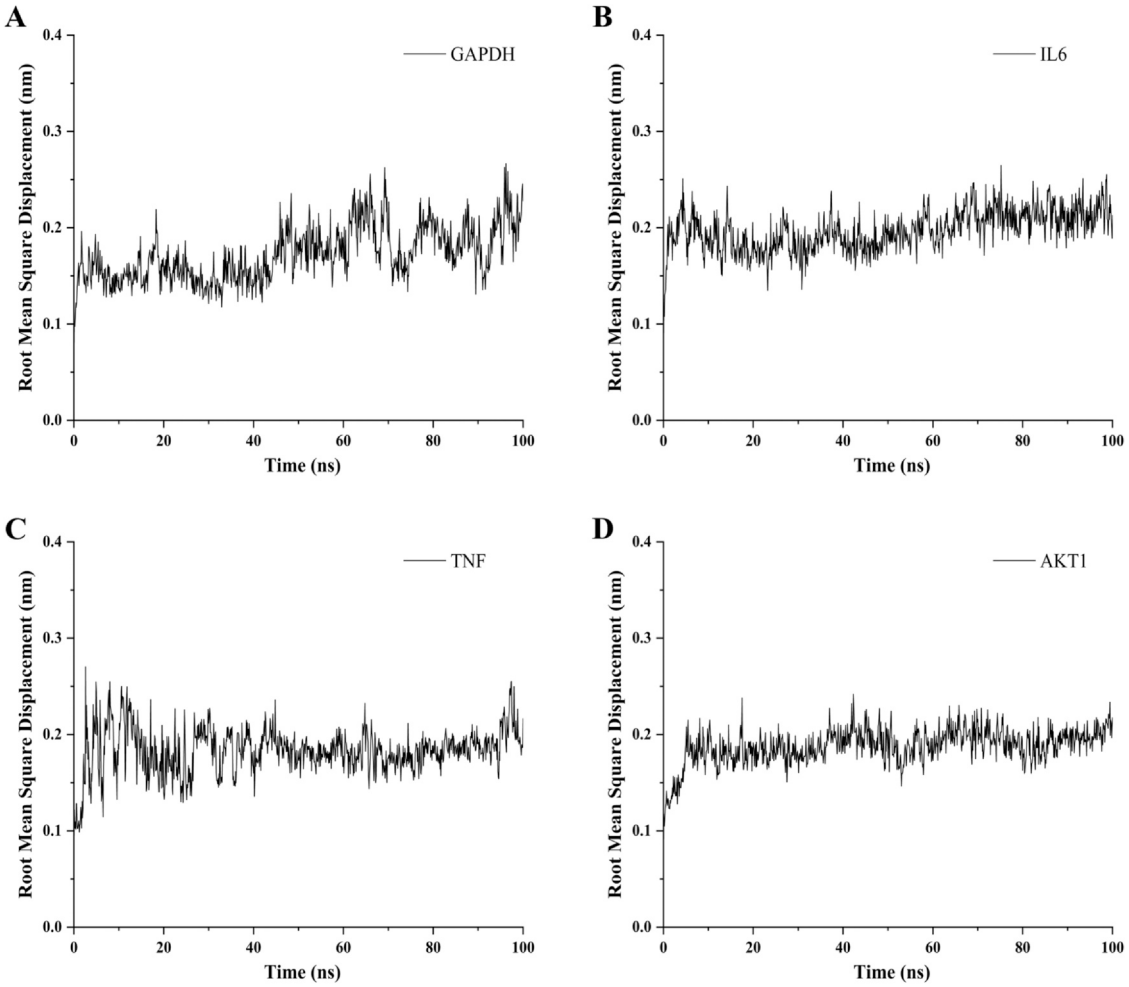

Fig. 9. RMSD Analysis of Complex Systems.

(Fig. 10D) complexes exhibited relatively smooth fluctuations. Specifically, the AKT1 complex maintained a stable structure throughout the simulation, with minimal positional changes in its amino acid residues. Residues such as LYS-158, ARG-206, LYS-301, ASP-302, ARG-370, and ARG-465 in the AKT1 protein exhibited relatively active states, suggesting that these residues may play a key role in the stability of the complex.

Fig. 11 compares the Radius of Gyration (RG), which represents the average distance between the center of mass of all atoms in the protein-ligand complex and the center of mass of the entire complex, to characterize the compactness of the protein. The RG changes of the AKT1 complex were notably smaller and more consistent, indicating that AKT1 forms a more compact and stable complex with sitosterol, with strong binding and a well-formed structure.

Discussion

EMT is a hormone-dependent, chronic, and multi-systemic disease. Given the limitations and shortcomings of current treatments for EMT, there has been increasing research into the potential therapeutic benefits of TCM. In TCM, EMT is classified under the categories of “dysmenorrhea” and “abdominal pain”, with its underlying causes attributed to qi stagnation, qi deficiency, and liver and kidney deficiencies. The treatment approach for EMT in TCM focuses on benefiting qi, nourishing yin, and promoting blood circulation to remove blood stasis.[21] The six herbs in Kuntai capsule are known for their properties in tonifying essence and marrow, nourishing yin, and replenishing blood. These herbs are traditionally used to treat conditions such as premature ovarian failure, perimenopausal syndrome, and EMT.[22] In this study, the mechanism of action of Kuntai capsule for the treatment of EMT was explored using an integrated approach, combining network pharmacology, molecular docking, and molecular dynamics simulation methods.

In this study, the core components of Kuntai capsule for the treatment of endometriosis EMT were identified. The results revealed that key components, such as sitosterol, panicolin, and rivularin, are primarily derived from Rehmanniae Praeparata, Radix Paeoniae Alba, and Radix Scutellaria Baicalensis. Among them, sitosterol plays a therapeutic role by inhibiting the expression of inflammatory factors such as IL-6. It also exhibits biological activities, including antioxidant, estrogen-like, and antitumor effects, which help promote estrogen production by the ovary.[23] Additionally, flavonoids present in these herbs have been shown to possess immunomodulatory, tumor telomerase activity-inhibiting, and anti-inflammatory effects.[24] Flavonoids have also been demonstrated to reduce lesion size, alleviate pain, and decrease postoperative recurrence rates in experimental EMT models.[25]

By constructing the disease-component-target network and performing PPI network analysis, this study identified core targets involved in the treatment of endometriosis EMT with Kuntai capsule, including TNF, GAPDH, IL6, AKT1, and TP53. It was found that IL6 and TNF gene expression can affect the occurrence and development of EMT.[26] IL6 is a cytokine involved in inflammatory responses and immune regulation. Elevated levels of IL6 in the peritoneal fluid of EMT patients have been found to exacerbate inflammation and internal environment disturbances.[27] TNF plays a crucial role in promoting immune responses and inflammation, while also regulating follicular

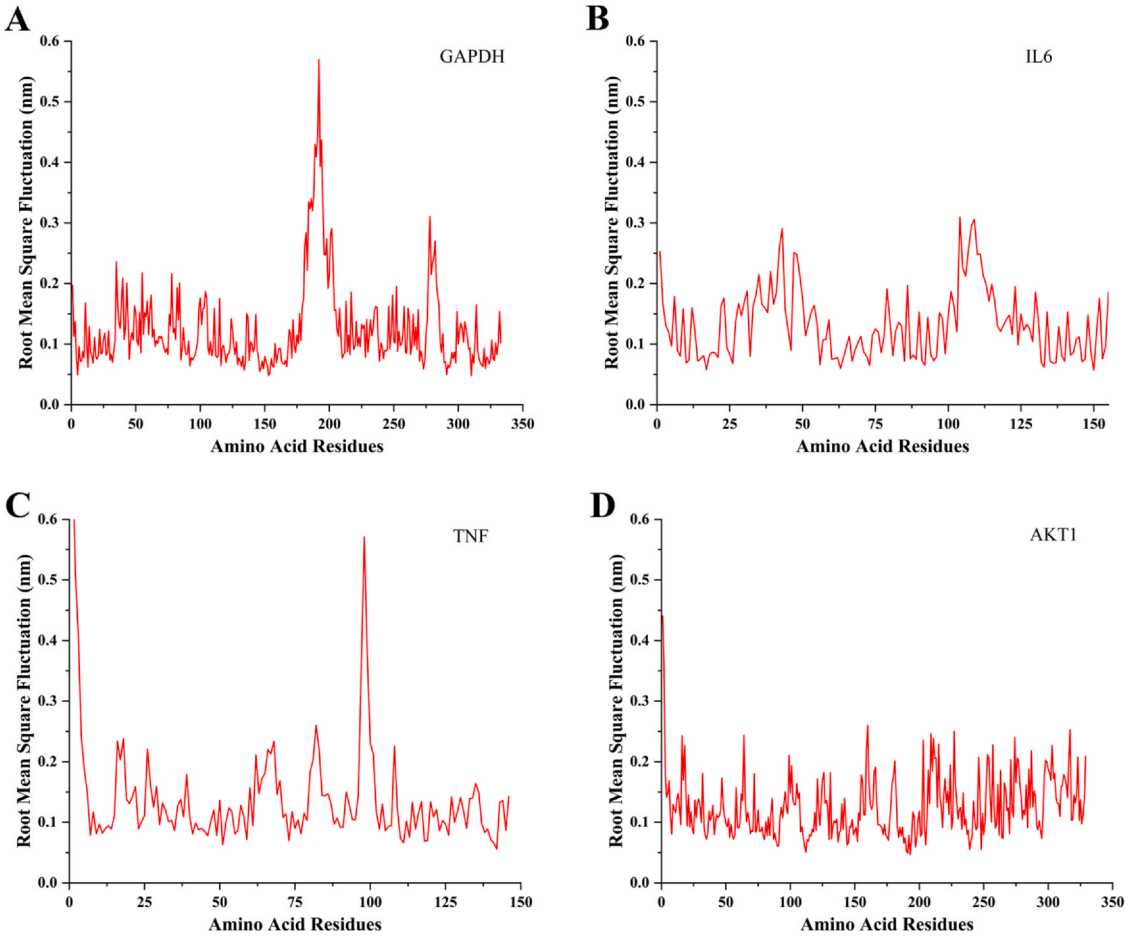

Fig. 10. RMSF Analysis of Complex Systems.

development and secretion.[28] AKT1, a key protein in the PI3K/Akt pathway, is involved in cell growth regulation and plays a role in endometrial stromal cell autophagy and invasion, thus contributing to the pathogenesis of EMT.[29] GAPDH is involved in oxidative stress-induced apoptosis of cycling cells.[30] TP53, a tumor suppressor gene, is critical for maintaining cell cycle regulation and genome stability, inducing cell differentiation and apoptosis. Mutations in TP53 are associated with highly aggressive and advanced cancers.[31] These findings highlight the significant roles of TNF, GAPDH, IL6, AKT1, TP53 and other targets in endometrial diseases, particularly in endometrial cancer and EMT. The results suggest that the targets of Kuntai capsule in treating EMT primarily involve in processes of anti-inflammation, hormone regulation, cellular autophagy, invasion inhibition, oxidative stress and anti-cancer.

The results of the GO and KEGG enrichment analyses show that the targets of Kuntai capsule in the treatment of EMT affect multiple biological processes, such as the negative regulation of apoptotic processes, response to xenobiotic stimuli, and the positive/negative regulation of gene expression. Kuntai capsule primarily modulates several key signaling pathways, such as pathways in cancer, proteoglycan-related pathway, PI3K/Akt pathway, and lipid and atherosclerosis signaling pathways, thereby exerting pharmacological effects on cell proliferation, survival, and apoptosis. Among them, oncogenic pathways such as PI3K/Akt are involved in focal angiogenesis, autophagy, and apoptosis of ectopic tissue cells. The PI3K/Akt signaling pathway has been identified to play a key role in EMT progression.[32] Inhibition of this pathway can reduce endometriosis development, alleviate EMT-induced pain, and mitigate fibrotic lesions in ectopic tissue. Furthermore, another study has suggested that triglycerides may be positively correlated with the severity of EMT, and that lipid and atherosclerosis signaling pathways may contribute to the onset and progression of endometriosis by influencing inflammation, angiogenesis, apoptosis, and immune regulation.[33] These findings collectively confirm that that Kuntai capsule exerts its therapeutic effects on EMT by modulating key apoptotic and other processes.[34]

To further validate the findings from the network pharmacological analysis, molecular docking and molecular dynamics simulations were conducted to assess the binding affinity and stability of the core components and targets. The molecular docking results revealed that 17 out of the tested groups had an affinity of $\leq$ -5, indicating that 68 % of the docking performance was good. This suggests that Kuntai capsule may exert its therapeutic effects on EMT through active components like sitosterol and flavonoids. The top 4 most tightly bound groups were selected for molecular dynamics simulations. Among these, the complex formed by AKT1 and sitosterol exhibited the best stability. The results, based on Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), and Radius of Gyration (RG), demonstrated a high binding affinity between AKT1 and sitosterol, alongside a stable and compact structure of the complex. These findings suggest that sitosterol is a highly bioactive compound with strong inhibitory potential against AKT1, both at static and dynamic molecular levels, providing a solid theoretical basis for the treatment of EMT with Kuntai capsule.

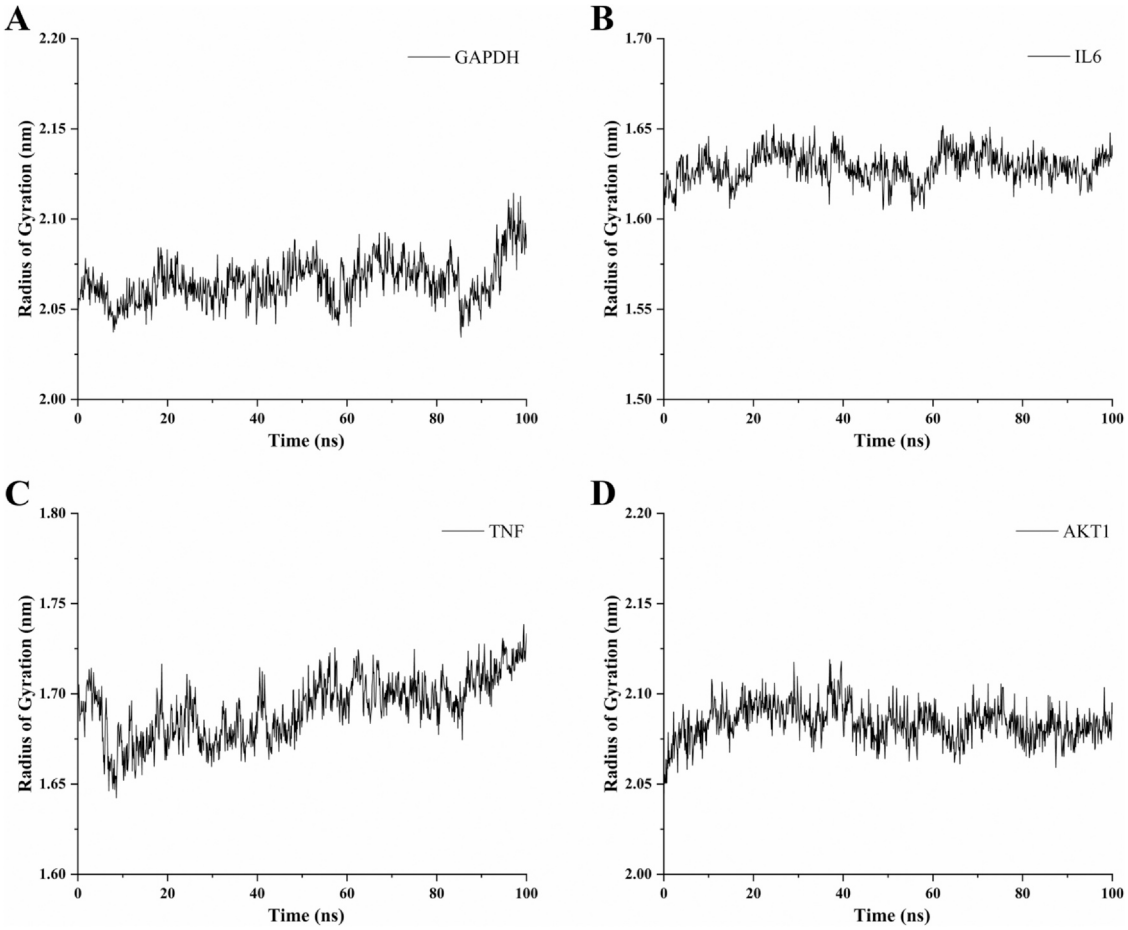

Fig. 11. RG Analysis of Complex Systems.

Conclusion

The mechanism of action of Kuntai capsule for the treatment of EMT is complex. The present comprehensively demonstrated that Kuntai capsule may improve treatment efficacy through its core components, such as sitosterol, panicolin, and rivularin, which acted on the multi-targets such as TNF, GAPDH, and IL6. The results of molecular docking and molecular dynamics simulations further confirmed the binding between the core components of Kuntai capsule and their respective targets, particularly the stable binding of sitosterol to AKT1, which maintains both conformation and activity. These findings support the hypothesis that sitosterol acts as a potential inhibitor of AKT1 in the treatment of EMT. This study provides a foundation for the mechanism of action of Kuntai capsule in treating EMT, offering valuable insights for future experimental research. However, as database-based studies may lead to biased results, further clinical and experimental investigations are necessary to validate these findings and to explore more specific therapeutic mechanisms.

Declarations

Not applicable.

Authors' contributions

Shanshan Li: Conceptualization, Methodology, Software, Data curation, Writing - Original draft preparation, Writing - Reviewing and Editing. **Longhui Shen:** Visualization, Investigation, Supervision, Validation.

Ethics approval and consent to participate

Not applicable.

Consent for publication

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Declaration of Competing Interest

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Authors' other information

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