Exploration of Underlying Mechanisms for Modified Xi-Xin-Tang III in Treating Alzheimer’s Disease through Network Integration Investigation

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Research Article

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Abstract

Background

Chinese natural herbal formula modified Xi-Xin-Tang III (mXXTIII) exhibits therapeutic effects for Alzheimer's disease (AD). This study aimed to explore the underlying mechanism of mXXTIII for treating AD and discover the main active ingredients and potential drug targets through a combination of network pharmacology-based strategy and molecular docking technique.

Methods

Single herbs in mXXTIII were screened for active ingredients, and target proteins were predicted. Target screening for AD was performed to establish a disease target database. Subsequently, a protein-protein interaction network was constructed and the correlation between proteins in the network was used to obtain gene clusters, export the subnetwork, and analyze the biological processes facilitated by the targets in this subnetwork. GO and KEGG enrichment analyses of key genes were conducted using the DAVID database. Finally, molecular docking of critical targets and active ingredients was conducted and their interaction patterns were visualized.

Results

The research received 81 active ingredients, 519 targets, and 3089 disease targets. A total of 264 potential targets of mXXTIII against AD were identified by drawing a Venn diagram and the top action pathways were recognized according to GO and KEGG enrichment analysis.

Conclusion

Multiple active ingredients, targets, and pathways may be involved in intrinsic molecular actions of mXXTIII in the recovery of AD. The major active ingredients (quercetin, baicalein, formononetin, etc.), critical targets, and key pathways could have played more important roles. The findings may provide a reference for further studies and assessments on the mechanism of resisting AD.

Introduction

Alzheimer's disease (AD) is a chronic neurodegenerative disorder characterized by cognitive decline, decreased ability for performing daily life activities, and psycho-behavioral abnormalities (Querfurth and LaFerla, 2010). It is prevalent in one-tenth of individuals over the age of 65 years (Pietrzak et al., 2015; Knopman et al., 2021). Due to an aging population, the incidence of AD and related dementia is on the rise. The World Alzheimer's Disease Report 2020 reveals that a new case of AD is diagnosed every 3 seconds worldwide (REPORT, 2020). AD is a serious health concern; however, the mechanism underlying
its progression and onset remains unclear. Currently, there are no effective drugs that can block the progression of AD.

The studies and applications of traditional herbal medicine in numerous fields have attracted the interest of several researchers worldwide. A combination of plant species/minerals prescribed for improving efficacy and reducing adverse effects by practitioners of traditional Chinese medicine (TCM) based on clinical experience from ancient times, is referred to as a formula (Wang et al., 2008). Nearly 100,000 traditional formulae have been recorded in China, and there are many characteristic TCM theories for formulae known for the treatment of diseases, such as yin and yang (Fu et al., 2021), cold, hot, and neutral nature (Fu et al., 2017), syndrome and sign differentiation (Hu et al., 2011), modification of prescriptions (Li et al., 2014), and addition and subtraction (An et al., 2021). These formulae can be enhanced based on the above-mentioned theories for different individuals or disease scenarios (Liu et al., 2019).

Xi-Xin-Tang III (XXTIII) is a formula containing 9 kinds of herbs, namely, Pinellia ternata, tangerine peel, Poria cocos, Aconite root, licorice root, ginseng, ShenQu, Acorus calamus, and zizyphus jujuba, which was first documented in the “Bian Zheng Lu (Records for Syndrome Identification) of the Qing Dynasty.” It has been widely used to treat AD patients in China and is considered to prevent disease progression and ameliorate cognitive function by inhibiting tau phosphorylation pathology (Diwu et al., 2013a; b). Modified Xi-Xin-Tang (mXXTIII) is processed based on XXTIII and a herb, roasted Radix Polygalae (Deng et al., 2020), and shows significant clinical efficacy. However, its molecular mechanism of action lacks comprehensive and systematic elucidation owing to the involvement of numerous components and complex intermolecular relationships. Therefore, further research is required.

Network pharmacology consists of traditional and analytical bioinformatics and systems medicine. Network pharmacology has been applied in TCM research and for further drug discovery in recent years (Nogales et al., 2022), and it can reflect the features of multiple components and targets of TCM (Du et al., 2022; Zhang et al., 2022). Molecular docking is a technique that is performed based on the characteristics of the receptor and the mode of interaction between the receptor and the ligand. Existing evidence suggests that molecular docking is fundamental in the field of computer-assisted drug research (Saikia and Bordoloi, 2019). The study aimed to explore the underlying mechanism of mXXTIII for anti-AD effects and expound on its main active ingredients, potential targets, and action pathways using network pharmacology and molecular docking. The flow chart of the study is shown in Supplementary Figure.

Materials and Methods

Component Composition

The formula mXXTIII comprises 10 kinds of Chinese herbs, including Pinellia ternata, Citrus sinensis, Poria cocos, Aconite root, Glycyrrhiza uralensis, Ginseng, ShenQu, Acorus calamus, Zizyphus jujuba, and roasted Radix Polygalae. TCMSP (https://tcmspw.com/tcmsp.php/), TCMID
(http://www.megabionet.org/tcmid/), and other databases related to Chinese medicine ingredients were used to obtain information on pharmaceutical ingredients, including the type and quantity, molecular name, and molecular mass of ingredients in every single herb. The structures of all the ingredients were imported into PubChem (https://pubchem.ncbi.nlm.nih.gov/)(Kim et al., 2019) for querying and normalization. The corresponding PubChem CIDs were obtained and the SDF structures were downloaded.

Screening the Active Ingredients of TCM

ADMET of drugs refers to their absorption (A), distribution (D), metabolism (M), excretion (E), and toxicity (T). It comprises pharmacokinetics and is typically used in contemporary drug design and screening. In this study, the ADMET Descriptors module of Discovery Studio 2017 R2 was employed to predict these parameters for ingredients in mXXTIII, and all the active ingredients in this formula were screened. Screening the Active Ingredients of TCM based on human intestinal absorption (ADMET-Absorption-Level and ADMET-Aqueous-Solubility). Chemical compounds with ADMET-Absorption-Level of 0, 1, and 2, and ADMET-Solubility-Level of 1, 2, 3, and 4 were included and analyzed.

Prediction of Target Proteins of Active Ingredients in mXXTIII

The following two steps were adopted for the prediction of the target proteins of the active ingredients in Chinese herbal medicines: first, the smiles structures of the screened active ingredients were entered into a Drug bank (https://go.drugbank.com/), Therapeutic Target Database (http://db.idrblab.net/ttd/) and SwissTargetPrediction platform (http://www.swisstargetprediction.ch/) to predict the relevant targets of the active ingredients in the herbs, and the targets in “Homo sapiens” were selected for further screening. Thus, a database of active ingredient–targets of mXXTIII was constructed.

Second, target prediction was performed following the methods of Fu et al.(Fu et al., 2017), which show higher molecular energy states lead to lower average molecular weights and greater correlation with TCM’s hot-warm characteristics, while the lower molecular energy state, the greater the correlation with TCM’s cold-cool characteristics. Additionally, cold-related compounds were found to have sedative properties and to be related to “mental and behavioral disorders” (Fu et al., 2017). Deep learning and Bayesian network algorithms were utilized to test and score the active ingredients in mXXTIII and all targets and their network topology parameters were calculated according to the scoring criteria to select the targets of the active ingredients in mXXTIII for subsequent analysis.

Disease Target Screening

Disease-related targets were obtained by retrieving information from the following databases: GeneCards database (http://www.GeneCards.org/), DisGeNet database (http://www.disgenet.org/), Therapeutic Target Database database(Zhou et al., 2022) (http://db.idrblab.net/ttd/), and OMIM database (https://www.omim.org/) with “Alzheimer’s disease” as the search term. Genes with a score greater than 5.657 were screened from the Gene Cards database. DisGeNet was used to search genes whose source
was the CTD-human database (Davis et al., 2021). Disease-related genes were extracted from the OMIM and Therapeutic Target Database databases. The data obtained from the four databases were combined and their intersection was taken following the removal of duplicate or invalid genes to establish the “disease target database”.

**Protein-Protein Interaction Network and Screening of Hub Genes**

The STRING database (https://string-db.org/) was used to construct protein-protein interaction (PPI) network for the common active ingredients and disease targets following intersection analysis. Specifically, the intersections for each active ingredient target of mXXTIII and disease targets of AD were taken and uploaded to the STRING database. The biological species was set as “Homo sapiens” and confidence was $\geq 0.400$. The constructed PPI network was imported into the Cytoscape 3.8.2 software. The topological algorithm based on the cytoHubba plugin was utilized to predict important protein nodes and subnetworks in the network. In this study, the hub genes were screened based on five parameters – DEGREE (Degree Correlation), MNC (Maximum Neighborhood Component), MCC (Maximal Clique Centrality), EPC (Edge Percolated Component), and CLONESS (Closeness Centrality) and visualized. Cluster analysis was performed using the MCODE plugin. The gene clusters were identified based on the correlation between proteins in the network and subnetworks were exported. The differential genes in each gene cluster were extracted and the main biological processes of these targets in the subnetworks were analyzed.

**Functional and Disease Enrichment Analysis**

GO functional annotation is used for large-scale functional enrichment studies of genes, including biological processes (BP), molecular functions (MF), and cellular components (CC). KEGG is a widely used database with information on genomes, biological pathways, diseases, and drugs. The screened hub genes were imported into the DAVID 6.8 database (https://david.ncifcrf.gov/), and the species was set as “Homo sapiens”. Subsequently, GO and KEGG pathway analysis ($P < 0.05$) was performed to analyze the relevant BPs and signaling pathways related to the key targets. The Cytoscape 3.8.2 software was used for visualization.

**Active Ingredient – Potential Target – Pathway of Action Network**

The Cytoscape 3.8.2 software was used to establish the “ingredient-target-pathway” network for mXXTIII. The network consisted of three parts–active ingredients, target proteins, and action pathways corresponding to mXXTIII. It was used to analyze and elucidate the mechanism of action of multiple ingredients, targets, and pathways in the treatment of AD.

**Molecular Docking for Critical Targets and Ingredients**

Molecular docking was conducted using CDOCKER in the Receptor-Ligand International module of the software Discovery Studio 2017 R2. Precise docking and analysis of key targets and major ingredients of Chinese herbs obtained from the above network were performed. The 3D structures of small molecule
chemical compounds of the main active ingredients of Chinese herbs were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/) according to their PubChem-ID numbers and imported into Discovery Studio 2017 R2. The high-resolution crystal structures of the key targets were downloaded from the Protein Data Bank (PDB) database (http://www.rcsb.org/pdb/home/home.do). The active site of each protein is centered on the active amino acid site of the original ligand action marked in the crystal structure to construct the corresponding “active pocket”. Consequently, the system was utilized to search for “active pocket” in the vicinity of the active site and finally locate the “active pocket” for the target. The CDOCKER parameter settings were as follows: the pose Cluster Radius was set to 0.5; random conformations to 10; orientations to refine to 10, and the remaining default parameters were unchanged.

Results

Screening of Active Ingredients in mXXTIII

A total of 212 chemical compounds were identified in mXXTIII from the data obtained from relevant databases. The main structural types included phenylpropanoids, flavonoids, alkaloids, sterols, and terpenoids. The formula comprised several chemical compounds, and the DS software was used to predict the ADMET parameters of the chemical compounds contained in the formula based on their chemical structure to identify the possible active ingredients. Finally, 170 active ingredients were screened, including 4 in Pinellia ternata, 4 in Tangerine peel, 16 in Fushen, 10 in Aconite, 79 in Licorice, 14 in Ginseng, 9 in Shenqu, 3 in Acorus tatarinowii, 6 in wild Jujube kernel, and 25 in roasted Polygala. Detailed information on the selected active ingredients is provided in Supplementary Table S1. Some common active ingredients were identified. Both GC15 and SZR1 are betulinic acid (Pubchem ID:64971); GC47, RS9, and SCP3 are kaempferol (Pubchem ID: 5280863); GC66 and RS10 are maackiain (Pubchem ID: 91510); GC72, YZ21, and SQ6 are quercetin (Pubchem ID: 5280343); SZR4 and SQ2 are daucosterol (Pubchem ID: 5742590), and YZ18 and FS9 are palmitic acid (Pubchem ID: 985).

Screening of Targets in mXXTIII Associated with AD

The targets with a p-value > 0.9 were screened as “targets of active ingredients” based on the prediction results, and a total of 519 potential action targets of active ingredients were obtained for mXXTIII. The number of disease targets in the Gene Cards database was higher for “Alzheimer’s disease” and 2801 targets were selected with a score > 5.657. A total of 200 disease targets were obtained from the OMIM database, 385 from the DisGeNET database, and 143 in the TTD database. Finally, 3089 disease targets were obtained after merging and removing the duplicates. The intersection of these 519 potential action targets with 3089 disease targets was obtained from the Venn diagram (Fig. 1A). A total of 264 potential action targets of mXXTIII for the treatment of AD were identified and the herb-ingredient-target interaction network was constructed (Fig. 1B).

Construction of the PPI Network and Screening of Critical Targets
The screened potential targets were input into STRING to obtain the information on PPIs, which was imported into Cytoscape to construct the PPI network. A total of 264 nodes (target proteins) and 4194 edges (protein-protein interactions) were obtained (Fig. 2A). In the interaction network, the node size and color demonstrate the node degree value. The larger the node and the darker the red, the larger the corresponding degree value, indicating that several predicted disease-related targets can interact effectively with that target. MNC, DEGREE, MCC, CLONESS, and EPC parameters were used for screening (Fig. 2B). The hub genes were selected by calculating and analyzing the network structure and the weighted connection between nodes. Intersections of the top 30 results of each algorithm (Supplementary Table S2) were taken to obtain 22 key targets (Fig. 2C, Supplementary Table S3). The top two key targets were AKT1 and MAPK3.

**Subnetwork Structure**

MCODE subnetwork analysis can identify clusters or genes that are closely linked in the network. The point with the highest weight obtained by weighted computation was set as the “seed”. Starting from the “seed” and recursively moving outwards, nodes joining the subnetwork were identified. The closer the target was to the center, the greater its importance. Subnetwork 1 is centered on CXCL8 to which critical connected targets include PTGS2, AR, and RELA (Fig. 3A). The core of subnetwork 2 is NFKB2 to which critical connected targets include GSK3B, ERBB2, and ESR1 (Fig. 3B). The core of subnetwork 3 is TUBA1B to which critical connected targets include TUBB2A, and GABRB3 (Fig. 3C). Significant targets of sub-network 4 are COMT, ACHE, and ADRA2A (Fig. 3D). The core of sub-network 5 is CDK2 to which critical connected targets include NOX4, PLCG1, and EGFR1 (Fig. 3E).

**GO Annotation**

To examine the functional distribution of key targets, 264 key targets were queried on the DAVID 6.8 database for GO enrichment analysis (Fig. 4, Supplementary Table S4). A total of 745 BP terms, 190 MF terms, and 91 CC terms were screened. Combined with the literature review, the key targets according to BP terms were enriched in neural nucleus development, negative regulation of axonogenesis, embryonic pattern specification, and pigment biosynthetic process; the key terms in CC were enriched in contractile actin filament bundle, stress fiber, histone methyltransferase complex, and spindle microtubule, and the key MF terms were enriched in neural nucleus development, embryonic pattern specification, negative regulation of axonogenesis, pigment biosynthetic process, and mechanoreceptor differentiation.

**KEGG Pathway Analysis**

KEGG pathway enrichment analysis of potential targets was conducted on the DAVID 6.8 platform (P < 0.05) (Fig. 5, Supplementary Table S5). The top 10 signaling pathways were HIF-1, cAMP, ErbB, Neurotrophin, Gap Junction, Vascular endothelial growth factor (VEGF), Thyroid Hormone (TH), Dopaminergic Synapse, Long-term Potentiation (LTP), and Cholinergic synapse cascades. These results suggested that the key targets of mXXTIII exerted their anti-AD effects through the above signaling pathways.
**“Ingredient - Target - Pathway” Network Construction**

The single herb, ingredients, potential targets, and the screened signaling pathways in mXXTIII were imported into Cytoscape 3.8.2 to construct the “herb-ingredient-target-pathway” diagram (Fig. 6A). The MCC algorithm of cytoHubba was used to calculate the closest correlation between ingredients and key targets (Fig. 6B).

**Molecular Docking of Key Targets and Related Ingredients**

The low binding energy of the receptor-ligand binding conformation in molecular docking signifies that the conformation is stable and therefore the most likely interaction. The docking of key target genes and their related ingredients revealed negative binding energies, implying that the related ingredients could bind well to the targets (Supplementary Table S6). Formononetin had the lowest docking binding energy of -8.43 kcal/mol to the target RELA, and the values for quercetin-GSK3B, formononetin-NFKB2, gancaoninA-MARK1, lanierone-AR, lanierone-CXCL8, formononetin-MARK1, baicalein-MARK1, baicalein-TP53, and baicalein-NFKB2 were all lower than −6 kcal/mol. The binding pattern is shown in Fig. 7.

**Molecular Docking of Classical Therapeutic Targets and Representative Ingredients**

Aβ, BACE1, and AchE are commonly regarded as the classical therapeutic targets in AD. The drug target binding affinity and the best-scored docked position between 5 representative ingredients (quercetin, baicalin, formononetin, lanierone, and gancaoninA) and these therapeutic targets are indicated in Supplementary Table S7. Most of them showed strong associations and stable conformation (Fig. 8).

**Discussion**

AD is a complex degenerative disease with no known cure. Globally, researchers are making numerous attempts in the field. Formulae of TCM including XXTIII and mXXTIII are widely used to treat AD (Howes et al., 2017; Ma et al., 2022; Zhang et al., 2022) and their efficacy has been confirmed in several experiments (Diwu et al., 2013a; b). Owing to multiple ingredients, targets and action pathways of formulae, the specific molecular biology of mXXTIII against AD remains unclear. In this study, network pharmacology and molecular docking were comprehensively used to investigate the mechanism underlying the therapeutic effects. Network pharmacology was used to construct the “herb-active ingredient-potential target-action pathway-disease” network for the treatment of AD with mXXTIII. A total of 81 main active ingredients, 264 potential targets, 22 critical targets, and 5 important subnetworks were obtained; the HIF-1 signaling pathway was critically affected by this formula. The results of molecular docking indicated good binding of all key target genes to their related ingredients, with docking binding energy of 10 pairs of active ingredient-target less than −6 kcal/mol. The lowest docking binding energy was for formononetin binding to RELA.

GO and KEGG analysis suggested that mXXTIII was involved in the treatment of AD through several BPs and multiple signaling pathways. The key terms in GO analysis were enriched in nuclear development,
negative regulation of axonogenesis, embryonic patterning specification, contractile actin bundles, and stress fibers. The top-ranked signaling pathways according to KEGG analysis were the HIF-1, cAMP, ErbB, Neurotrophic Factor, Gap Junction, and VEGF transduction cascades. The results suggested that the therapeutic mechanism of this formula was predominantly related to the above multiple biological functions and signaling pathways.

Most of the 22 key therapeutic targets identified in the study are involved in apoptosis, DNA damage, immune regulation, and anti-oxidation, including the top two– AKT1 and MAPK3. The protein of AKT1 gene encoding is a protein kinase B that plays a critical regulatory role in various cellular processes such as glucose metabolism, apoptosis, cell proliferation, and cell migration(Emamian et al., 2004). Activation of the AKT signaling cascade has been found to inhibit tau phosphorylation, thus improving AD(Wang et al., 2018). MAPK3 gene encodes the protein Mitogen-Activated Protein Kinase 3, which can mediate intracellular signal transduction and a variety of biological functions, such as cell growth, adhesion, survival, and differentiation, and participate in the response to potentially harmful abiotic stress stimuli (high osmotic pressure, oxidative stress, DNA damage, low osmotic pressure)(Lu and Malemud, 2019). It was reported that activation of MAPK3 could cause hyperphosphorylation of tau protein in neurons, and thus contribute to the development of sporadic AD(Wisessaowapak et al., 2021). These results support the possibility that two key targets are involved in AD through protein interactions.

According to subnetwork analysis, five tighter subnetworks were identified. Subnetwork 1 was centered around CXCL8, and the important targets connected to it were all related to inflammation; subnetwork 2 was centered around NFKB2, and the targets connected to it were all related to the spindle microtubule. The core of subnetwork 3 was TUBA1B which was connected to essential targets related to microtubules. The significant targets of subnetwork 4 were COMT, ACHE, and ADRA2A, which was related to dopaminergic, acetylcholinergic, and adrenergic synapses and transmitter metabolism, implicating its importance in neurotransmission. The core of subnetwork 5 was CDK2, with important targets, including NOX4, PLCG1, and EGFR1, which are all related to the HIF-1 signaling pathway.

Finally, the visualization software was used to present the “ingredient-target-pathway” diagram. In addition, the closest connection was calculated utilizing the MCC algorithm of the cytoHubba plugin. Results revealed that HIF-1 signaling was the most critical action pathway. Thus, AD is closely related to chronic hypoxic conditions(Thakur et al., 2010). HIF-1 is a transcription factor responsible for cellular and tissue adaptation to low oxygen stress. Cells in a hypoxic state lead to hypometabolism and activate HIF-1, inducing the expression of a large number of genes related to hypoxia, thus allowing cell survival in a hypoxic environment and facilitating the return to normoxic levels(Semenza, 2000). In AD patients, hypometabolic brain regions trigger overexpression of amyloid precursor protein and decreased clearance of Aβ. Aβ and hypoxia cause inflammation, oxidative stress, and finally neuronal death(Wang et al., 2021). Maintaining HIF-1 levels effectively attenuates nerve injury during hypoxia and delays the development of AD. Thus, HIF-1 is a neuroprotective agent in AD(Ashok et al., 2017). HIF-1 is also a potential drug target against neurodegenerative diseases, and the HIF-1-mediated pathway is essential to
the development of AD (Ogunshola and Antoniou, 2009). These results provide directions for further in-depth studies.

Molecular docking showed fine binding of key target genes to their related ingredients, with the most stable conformation between formonononetin and RELA. Other relatively stable pairs of ingredient-target with binding energies less than −6 kcal/mol were baicalein to MARK1, TP53, and NFkB2; formonononetin to NFkB2 and MARK1; lanierone to AR, CXCL8; quercetin to GSK3B, and gancaonin A to MARK1. In addition, the underlying mechanisms of mXXTIII improving the cognitive function were possibly associated with the high binding affinity between the above 5 ingredients and Aβ, BACE1, and AchE. Therefore, these active ingredient targets were notably related to the therapeutic effects of mXXTIII against AD, worth further study.

Previous studies have also corroborated that the representative active ingredients of single herbs in this formula, such as quercetin and baicalein, can exert therapeutic effects against AD through different pathways. Quercetin, a common flavonoid, is one of the most potent antioxidants in plants, which protects neurons from oxidative damage and improves cognition (Babaei et al., 2018; Khan et al., 2019; Zhang et al., 2020). Oral administration of quercetin nanoparticles in AD animal models can reduce neuronal degeneration and the formation of senile plaques and neurofibrillary tangles (Rifaai et al., 2020). Known bioinformatics analysis and molecular docking studies showed that quercetin had a good affinity with AchE active sites (Islam et al., 2013), which was later confirmed by in vivo experiments (Nazir et al., 2018). In addition, in vitro and molecular docking studies showed that quercetin had an effective anti Aβ aggregation activity (Espargaró et al., 2017). Some computer researches also showed that quercetin and CALHM1 (Khare et al., 2022), TNF-α (Ma et al., 2020), MAPK1 (Ma et al., 2020), Ferritin (Shamsi et al., 2021), TXNIP (Zhang et al., 2021), showed good or better docking. Baicalein is an important flavonoid. Evidence suggests that it is an effective neuroprotective agent (Sowndhararajan et al., 2017). Baicalein may inhibit tau aggregation by initializing the formation of nontoxic tau oligomers or sequestering oligomers (Gu et al., 2016; Sonawane et al., 2019), and can prevent Aβ-induced hippocampal LTP impairment by activating serine-threonine kinase phosphorylation (Gu et al., 2016). Baicalein also inhibits 12/15 LO, and GSK3B activity, decreases β-secretase (BACE1) and total Aβ concentrations and restores synaptic plasticity and memory deficits in mouse models of AD (Gu et al., 2016). In addition, baicalein improves behavioral dysfunction in AD rats (Zhou et al., 2016). Existing studies utilizing molecule docking showed that baicalein had strong BACE1 and AChE inhibitory properties (Han et al., 2019), and a high binding affinity with Aβ (Guo et al., 2022). Formonononetin is also a flavonoid ingredient, which has been found to exert protective effects against cardiovascular disease (Machado Dutra et al., 2021). Formonononetin can significantly reduce atherosclerosis by activating AMPK to regulate KLF4-SRA signaling in apoE-/-mice (Ma et al., 2020; Fan et al., 2022). Our research found that the combination of formonononetin and RELA has the most stable conformation, which maybe opens a window for us to study the role of the two in AD. Lanierone is a previously isolated and identified pheromone component (Teale et al., 1991). The information on this compound is scarce but the relationship between pheromones and AD possibly be a “blue ocean” (Frey, 2003). No relevant studies on Gancaonin A have been reported.
The study reveals the potential mechanism of mXXTIII in treating AD, which demonstrates important findings but has some limitations. First, the results of this study were in silico predictions without subsequent molecular biology experiments to validate them. Second, the complex composition of formulae, the effects of interactions between active ingredients, in vivo pharmacokinetics, and the intensity of modulation on each target could not be entirely included in our calculations, and thus, the exact mechanism needs to be studied in detail. The biological modulation of AD by this formula needs to be further assessed to seek novel ideas for developing novel drugs and identifying new targets for anti-AD effects.

Conclusion

Based on network pharmacological investigation, we identified the main active ingredients, key targets, and action pathways of mXXTIII for the treatment of AD. The HIF-1 signaling pathway was critical for treatment using this formula. However, this formula can produce marked effects through combining multiple targets, ingredients, and pathways. Using molecular docking, the combination of key target genes and their related ingredients were found to exhibit stable conformations. It also reflects the characteristics of the multiple active ingredient-target of this formula and points to a new direction for the study of the pathways and mechanisms underlying the development and progression of AD. The integration of network pharmacology and molecular docking is in line with TCM research theories and provides a scientific explanation for the combined actions of formulae. Our study also lays the foundation for further research on the intricate mechanism of mXXTIII for the recovery of AD. We would conduct a lot of in vivo or in vitro experiments to specifically verify the results of this study in the future.

Declarations

Authors’ contributions

ZK: data acquisition, analysis, writing, and editing of the manuscript; ZH, B-SF: Composition and analysis guidance and evaluation of compounds, and editing of the manuscript; W-YY, Z-DL: data acquisition and analysis; L-JY: Selection of Chinese herbal formula, study design, data analysis, and completed drawing; W-XY: Decision of Chinese herbal formula, data analysis, and data interpretation; W-YW: Decision of Chinese herbal formula, study design, data interpretation and critical revision of the manuscript. All authors read and approved the final manuscript.

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Competing Interests
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data Availability

The datasets presented in this study can be found in online repositories.

**Ethics approval**

This article does not contain any studies with human participants or animals performed by any of the authors.

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**References**


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**Figures**

**A**

AD

2825

264

255

**B**

mXXT

Figure 1

Interaction network diagrams of mXXT for treating AD. A. Venn diagram of action targets and disease targets of mXXT; B: mXXT’s herb-ingredient-target interaction network.

*The green hexagon represents the Chinese herb; the round represents the ingredient; and the blue diamond represents the target.*
Figure 2

Construction of PPI (protein-protein interaction). A. Interaction network of all target proteins. B. Venn diagram of top 30 target results from five algorithms MNC, DEGREE, MCC, CLONESS, EPC. C. Interaction network of key targets.
Figure 3

Figure 4

GO enrichment analysis of key targets. A. GO-BP analysis results of key targets. B. GO-CC analysis results of key targets. C. GO-MF analysis results of key targets.
Figure 5


Figure 6


*The green hexagon is the name of a single Chinese herb, the circle is the ingredient; the blue diamond is the target; and the purple arrow is the name of the pathway.
Figure 7

3D and 2D simulation diagrams of molecular docking between key targets and compounds. A. 3D and 2D diagrams of docking between Target NFKB2 and formononetin. B. 3D and 2D diagrams of docking between Target RELA and formononetin. C. 3D and 2D diagrams of docking between Target MAPK1 and gancanin A. D. 3D and 2D diagrams of docking between AR and lanierone. E. 3D and 2D diagrams of docking between Target CXCL8 and lanierone. F. 3D and 2D diagrams of docking between Target MAPK1 and formononetin. G. 3D and 2D diagrams of docking between Target MAPK1 and baicalein. H. 3D and 2D diagrams of docking between Target GSK3B and quercetin. I. 3D and 2D diagrams of docking between Target TP53 and baicalein. J. 3D and 2D diagrams of docking between Target NFKB2 and baicalein.
Figure 8

Simulation diagrams of molecular docking between critically AD related targets and compounds. A. 3D and 2D diagrams of docking between Target Aβ40 and baicalein. B. 3D and 2D diagrams of docking between Target Aβ40 and formononetin. C. 3D and 2D diagrams of docking between Target Aβ40 and quercetin. D. 3D and 2D diagrams of docking between Target BACE and baicalein. E. 3D and 2D diagrams of docking between Target BACE and formononetin. F. 3D and 2D diagrams of docking between Target
BACE and gancanin A. G. 3D and 2D diagrams of docking between Target BACE and lanierone. H. 3D and 2D diagrams of docking between Target BACE and quercetin. I. 3D and 2D diagrams of docking between Target AchE and baicalein. J. 3D and 2D diagrams of docking between Target AchE and formononetin. K. 3D and 2D diagrams of docking between Target AchE and quercetin.

Supplementary Files

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