The network pharmacology study and molecular docking to investigate the potential mechanism of Acoritataninowii Rhizoma against Alzheimer’s Disease

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Abstract
Alzheimer’s Disease is considered as an insidious neurodegenerative progressive disease but its pathogenesis has not been elucidated. Acoritataninowii Rhizoma exhibits anti-dementia effects as a traditional Chinese medicine (TCM), which is linked to its anti-Alzheimer’s Disease mechanism. In this study, network pharmacology and molecular docking were used to examine the potential of Acoritataninowii Rhizoma for Alzheimer’s Disease. In order to construct PPI networks and drug-component-target-disease networks, disease-related genes and proteins were gathered from the database. Gene ontology (GO), pathway enrichment (KEGG), and molecular docking were used to forecast the potential mechanism of Acoritataninowii Rhizoma on Alzheimer’s disease. Therefore, 4 active ingredients and 81 target genes were screened from Acoritataninowii Rhizoma, 6765 specific target genes were screened from Alzheimer’s Disease, and 61 drug-disease cross genes were validated. GO analysis showed that Acoritataninowii Rhizoma can regulate processes such as the protein serine/threonine kinase associated with MAPK. KeGG pathway analysis showed that the signaling pathways affected by Acoritataninowii Rhizoma were fluid shear stress and atherosclerosis, AGE-RAGE and other pathways. Molecular docking implied that the pharmacological influences of the bioactive constituents of Acoritataninowii Rhizoma (Cycloaartenol and kaempferol) on Alzheimer’s Disease may related to ESR1 and AKT1, respectively. AKT1 and ESR1 may be the core target genes of the treatment for Alzheimer’s disease. Kaempferol and Cycloartenol might be core bioactive constituents for treatment.

Keywords Alzheimer’s Disease · Acoritataninowii Rhizoma · Network pharmacology · Molecular docking · TCM

Introduction
Alzheimer’s disease (AD) is a progressive, neurodegenerative and insidious disease (Hogh 2017). Comprehensive dementia is clinically illustrated by memory impairment, executive dysfunction, aphasia, agnosia, visual-spatial skill impairment, etc. (Lane et al. 2018). In addition, there is still no precise therapy to combat Alzheimer’s disease (Chen 2018, Xiao and Xin (2014)) and the disease usually lasts 10 to 25 years. As a result of Alzheimer’s disease,
patients, their families, and the society are subjected to a great deal of economic and emotional pressure (Huang and Mucke 2012).

As for right now, piracetam, acetylcholine inhibitors, glutamate receptor antagonists, etc. are the main drugs used to prevent and alleviate AD. Tacrine has been discontinued now since it can produce a serious effect on digestive function (Manning 1994). Rivastigmine and galantamine can not only lead to indigestion but also result in dizziness, headaches, and other negative reactions. Donepezil is useful for mild to moderate patients. While these drugs are commonly used to treat Alzheimer’s disease (AD), their overall impact changes as the underlying neurodegenerative process changes (Briggs et al. 2016). Therefore, finding new treatments and biomarkers for AD is essential (Vaz and Silvestre 2020).

Acoritataninowii Rhizoma is commonly used as Fangxiangkaiqiao in Chinese medicine. Acoritataninowii Rhizoma owns pharmacological effects like sedation, antidepressant, anti-dementia, cardiomyopathy protection, stomach, antioxidant, cough and anti-fatigue, etc. Classic traditional Chinese prescription medicines own their chief ingredients in the cure of the central’s diseases nervous system. The chief ingredients also show superior pharmacological activity in the cardiovascular system, respiratory system, and digestive system. Related study on AD has been conducted by some scholars, and it is of great importance to further explore the mechanism of Acoritataninowii Rhizoma on AD by data mining (Deng et al. 2015, Mao et al. 2015). What’s more, the network pharmacology’s holistic view is correspond with the view of traditional Chinese medicine (Li and Zhang 2013). As a bioinformatics method, cyberpharmacology was widely used to analyze the underlying mechanisms between drugs and diseases, and predict possible targets and pathways for certain components in various diseases, even in psychiatric disorders that are closely related to signal transduction in recent years (Zhang et al. 2019; Chen et al. 2019; Li et al. 2020). In addition, bioinformatics resources such as molecular docking have been developed, providing more evidence for rapid systematic analysis (He et al. 2019; Wang et al. 2018). Molecular docking methods were designed to evaluate the binding potentials of proteins and ligands through network pharmacological analysis and prediction (Hsin et al. 2016). Thus, a pharmacological network binding molecular docking was employed in this study to determine the feasibility and reliability for treating AD and evaluate the effectiveness and potential molecular mechanisms of Acoritataninowii Rhizoma on AD.

The research was guided in the following steps and demonstrated in Fig. 1:

1. Retrieving the composition and corresponding targets of Acoritataninowii Rhizoma and AD-related targets from database;
2. Form a disease network of drug component targets and organize visual analysis;
3. By network analysis, construct key components, core objectives, signal paths, and top-level functions;
4. Obtain and confirm potential targets by docking with the molecule’s corresponding components and top target.

Fig. 1 The schedule in the present study was outlined as followed: (1) the ingredients of Acoritataninowii Rhizoma along with their corresponding targets and AD associated targets were searched and screened from databases; (2) The network of medicine-ingredients-targets-disease was constructed, visualized, and analyzed; (3) key ingredients, core targets, top functions and signal pathways were analyzed by PPI, GO, KEGG and the networks were constructed; (4) the valuable targets were obtained and validated via molecular docking with corresponding ingredients and the top targets.
Materials and methods

Collection and screening of active ingredients in Acoritataninowii Rhizoma

The active ingredients derived from Acoritataninowii Rhizoma were collected from the following databases: Bioinformatics Analysis Tool for Molecular mechanism of Traditional Chinese Medicine (BATMAN-TCM) (http://bionet.ncpsb.org/batman-tcm/), Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) database (https://tcmspw.com/tcmsp.php) and Traditional Chinese Medicines Integrated Database (TCMID) (http://www.megabionet.org/tcmid/) (Liu et al. 2019, Zhang et al. 2020).

Prior to target prediction, absorption, distribution, metabolism, and excretion (ADME) were used to screen compounds and remove inactive ingredients (Ru et al. 2014). Candidate compounds were selected based on their ability to be orally absorbed, utilized, and biologically active: molecular weight (MW) ≤ 500, oral bioavailability (OB) ≥ 30%, blood–brain barrier (BBB) ≥ -0.3 and drug similarity (DL) ≥ 0.18. After screening the targets were gathered based on bioactive ingredients (Tao et al. 2019). The gene symbols of targets were calibrated by UniProt database (http://www.uniprot.org/uploadlists/) (Guo et al. 2020). After the collection and screening, the network of medicine-ingredients-targets was constructed by Cytoscape3.7.2 software.

Genes collection for AD

Information related to AD target genes was collected from databases (Zeng et al. 2019), including PharmGKB (https://www.pharmgkb.org/), the Online Mendelian Genetic Database (OMIM) (https://omim.org/), the Degenerative Database (https://www.disgenet.org/), the Therapeutic Target Database (TTD) (http://db.idrblab.net/ ttd/), DrugBank Database (https://www.drugbank.ca/), GeneCards (https://www.genecards.org/). Only the “Homo sapiens” protein associated with AD were selected. The target genes’ name were also standardized in uniprot database.

Intersection of targets between active ingredients and disease

The genes associated with Acoritataninowii Rhizoma and AD were analyzed to identify overlapping targets between bioactive constituents and diseases, enabling to predict the pharmacological effects of Acoritataninowii Rhizoma on AD. Venn diagrams of the intersection were created, using the network analysis software Venny 2.1.0 (https://bioinfogp.cnb.csic.es/tools/venny/). The number of overlapping genes was computed automatically.

The network of medicine-ingredients-targets-disease construction

Based on the overlapping targets between disease and compounds, a drug-component-target-disease network was built by Cytoscape3.7.2 software, and the interaction between AD and Acoritataninowii Rhizoma was speculated. A drug-component-target-disease network was created, and the interaction between Acocritataninowii Rhizoma and AD was hypothesized. After the construction of the network, topological properties like “degrees” and “intersex centrality” were figured by “Network Analyze” in the software (Gu et al. 2013).

Protein–protein interaction (PPI) network analysis

Cytoscape3.7.2 software and STRING database (https://string-db.org/) were used to create a functional proteins’ network to better understand overlapping targets (Ma et al. 2019), in which the condition was selected as "Homo sapiens". The confidence score with correlation degree ≥ 0.400 (medium confidence), as the cut-off value, was set to obtain the network. The counts of interaction between each protein were computed.

Gene ontology (GO) enrichment analysis

GO enrichment analysis was a commonly way to build computational model to provide a logical framework for gene functions and annotations (Xie et al. 2018). R package cluster Profiler was used to perform GO enrichment analysis with the “Homo sapiens” setting and a threshold value of $p < 0.05$ (Wang et al. 2019). Changes of genetic functions were manifested in molecular function (genes that regulate molecular activity), cellular components (the relationship between gene function and cellular structure), and biological processes (biological procedures that organisms achieve through genetic programs). Furthermore, the number of genes in each GO function were computed to show the potential genetic function of Acoritataninowii Rhizoma resulting in AD. The network of Go enrichment was constructed by Cytoscape3.7.2 software.

Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis

The KEGG analysis could provide information on pathways of AD and the pharmacological effects of Acoritataninowii Rhizoma. R package clusterProfiler was also used for KEGG enrichment analysis and to merge KEGG pathways to construct
a functional and efficient pathway network (Palombo et al. 2020). The enrichment was performed in the conditions of "Homo sapiens", with a threshold value of \( p < 0.05 \). The figure of genes in each pathway to build a network of target pathways were figured by using Cytoscape3.7.2 software. Moreover, the major pathways’ mappings were also gathered.

**Molecular docking**

In the network pharmacological research, Molecular docking was used to verify ingredient-target association (Priya et al. 2015). The molecular structure of each ingredient in the work was obtained from the TCMSP database (https://tcmspw.com/tcmsp.php) and saved as a mol2 format file. The 3D structures of the target protein were downloaded from the RCSB PDB database (http://www.rcsb.org/) and saved as a PDB format file. Before the docking process, the ligand and protein were prepared in the autodock software. The crystal structure of target proteins were preprocessed, including the removal of water molecules, protonation hydrogenation, and Gasteiger charges calculation (Berman et al. 2000). The minimal energy conformation requires the structure of the ligand. Protein–ligand with the lowest binding energy is considered the most likely target.

Comparing the active drugs for targets, the protein–ligand was select as the target of binding which has the lowest binding energy and biggest possibility. The docking of a protein–ligand with the lowest binding energy was chosen as the binding’s target with the greatest possibilities. The 2D interaction and 3D structure diagram were visualized by Ligplot + v.2.2.4 and PyMol2.4.1 software. Protein–ligand complex and their position of amino acid residues were evaluated.

**Results**

**Potential targets in Acoritataninowii Rhizoma and AD**

With searching the database TCMSP, TCMID, and BATMAN-TCM, detailed information on 4 active ingredients with 81 target genes in Acoritataninowii Rhizoma were obtained (Table 1). In addition, the topological properties of the network construction based on the Acoritataninowii Rhizoma-ingredients-targets showed that the number of nodes and edges was 85 and 106, respectively (Fig. 2). The network centralization was 0.738 and the network density was 0.029. The ingredient and targets with a maximum degree were kaempferol (64) and NCOA2 (3) , PGH2(3), CALM1(3), respectively. By searching databases such as DrugBank, GeneCards, OMIM, and PharmGKB, a total of 6765 target genes were found to be AD-specific target genes. Results indicated that the pharmacological profiles of Acoritataninowii Rhizoma were involved in multiple compounds and target genes. Similarly, the pathology of AD was also associated with numerous target genes.

**The intersection of potential targets**

As shown in Fig. 3A, 61 overlapping genes were calculated by matching the gene between Acoritataninowii Rhizoma and AD. 61 overlapping genes were calculated, including ADRA1B, ADRA1D, AHSA1, AHR, AKT1, AR, BAX, BCL2, CALM1, CASP3, CCNA2, CDK1, CDK2, CHEK1, CYP1A1, CYP1A2, CYP3A4, DI01, DPP4, ESR1, F2, GABRA1, GABRA2, GSK3B, GSTM1, GSTP1, HAS2, HMOX1, HSP90AB1, ICAM1, IKKB, INSR, JUN, KCNH2, KCNA1, MAPK8, MAPK14, MMP1, NOS2, NOS3, NR3C2, NR1I2, PGR, PIK3CG, PIM1, PSMD3, PPP3CA, PRSS1, PRKACA, PPARG, RXRA, PYGM, RELA, SCN5A, STAT1, SLC6A2, SLC2A4, SLPI, TOP2B, VCAM1, XDH. Overlapping genes accounted for 0.9% in total, indicating that the influence of Acoritataninowii Rhizoma on AD were possibly associated with the above-mentioned multiple overlapping genes.

**Network construction of medicine-ingredients-targets-disease**

As shown in Fig. 3B and Table 2, a network of drug-components-target-diseases consisting of 67 nodes and 140 edges was constructed. In the Venn diagram, the network contained 4 bioactive constituents and 61 overlapping genes. Cytoscape3.72 software was used to analyze other key parameters such as network density (0.063), network centralization (0.888), the average number of neighbors (4.149), and network heterogeneity (2.218). The pharmacological effect of Acoritataninowii Rhizoma on AD was performed by the active ingredients and the target genes.

![Table 1](https://example.com/table1.png)
As shown in Fig. 4B, a PPI network was constructed and analyzed 61 overlapping proteins’ interactions, including ADRA1B, ADRA1D, AHS1A, AHR, AKT1, AR, BAX, BCL2, CALM1, CASP3, CCNA2, CDK1, CDK2, CHEK1, CYP1A1, CYP1A2, CYP3A4, DPP4, ESR1, F2, GABRA1, GABRA2, GSK3B, GSTM1, GSTP1, HMOX1, HSP90AB1, ICAM1, IKBKB, INSR, JUN, KCNH2, KCNMA1, MAPK8, MAPK14, MMP1, NOS2, NOS3, NRU2, NR3C2, PPARG, PPP3CA, PIM1, PYGM, PRSS1, PRKACA, PIK3CG, PGR, PSMD3, RELA, RXRA, SCN5A, SLPI, STAT1, SLC2A4, SLC6A2, TOP2B, VCAM1, XDH. 59 proteins have been established in the connection except for HAS2 and DIO1. The main parameters of the network were analyzed through the STRING database: the number of nodes (61), the number of edges (355), the average node degree (11.6), and
the average local clustering coefficient (0.555). Additionally, the degree of each node was constructed by GraphPad Prism 5.0 (version 2.0; GraphPad Software Inc., San Diego, CA) (Fig. 4A). The top 10 protein node degrees were AKT1, AR,CASP3, ESR1, HSP90AB1,JUN, MAPK8, MAPK14, NOS3 and RELA. These core proteins might interact to the pharmacological effects of Acoritataninowii Rhizoma on AD.

**GO enrichment analysis for target genes**

The GO enrichment analysis is utilized to further evaluate the function of overlapping target genes in three aspects: molecular function, biological processes, and cellular components. Based on the threshold value based on \( p < 0.05 \). The top 30 in GO enrichment from the three functions above have been obtained.
As illustrated in Table 3 and Fig. 5A, the top 5 terms in molecular function were: protein serine/threonine kinase activity, ubiquitin-like protein ligase binding, RNA polymerase II-specific DNA-binding transcription factor binding, ubiquitin-protein ligase binding, and DNA-binding transcription factor binding.

Besides, the number of nodes and edges were 59 and 122 respectively, as illustrated in Fig. 5B, which shows the

Table 3  Molecular function of GO analysis

| ID           | Molecular function                                    | P value | Count | Gene name                                      |
|--------------|-------------------------------------------------------|---------|-------|------------------------------------------------|
| GO:0004879   | nuclear receptor activity                             | 2.29E-10| 7     | PPARG/ESR1/AR/AHR/PGR/NR1I2/RXRA              |
| GO:0098531   | ligand-activated transcription factor activity         | 2.29E-10| 7     | PPARG/ESR1/AR/AHR/PGR/NR1I2/RXRA              |
| GO:0003707   | steroid hormone receptor activity                      | 8.24E-10| 7     | PPARG/ESR1/AR/NR3C2/PGR/NR1I2/RXRA           |
| GO:0004674   | protein serine/threonine kinase activity               | 2.65E-07| 11    | AKT1/GSK3B/MAPK14/MAPK8/CDK1/IKBKB/PRKACA/CDK2/PK3CG/CHEK1/PIM1 |
| GO:0044389   | ubiquitin-like protein ligase binding                  | 1.02E-06| 9     | KCNH2/GSK3B/RELA/JUN/STAT1/BCL2/PRKACA/HSP90AB1/SCN5A |
| GO:0031072   | heat shock protein binding                             | 3.34E-06| 6     | CDK1/BAX/AHR/CYP1A1/HSP90AB1/AHSA1            |
| GO:0061629   | RNA polymerase II-specific DNA-binding transcription   | 4.68E-06| 8     | PPARG/GSK3B/ESR1/MAPK14/JUN/STAT1/RXRA       |
| GO:0031625   | ubiquitin protein ligase binding                       | 6.55E-06| 8     | KCNH2/GSK3B/REL/JUN/BCL2/PRKACA/HSP90AB1/SCN5A |
| GO:0020037   | heme binding                                          | 6.94E-06| 6     | CYP3A4/CYP1A2/NOS3/HMOX1/NOS2/CYP1A           |
| GO:0046906   | tetrapyrrole binding                                  | 1.05E-05| 6     | CYP3A4/CYP1A2/NOS3/HMOX1/NOS2/CYP1A           |
| GO:0016725   | oxidoreductase activity, acting on CH or CH2 groups    | 1.09E-05| 3     | CYP3A4/CYP1A2/XDH                            |
| GO:0016705   | oxidoreductase activity, acting on paired donors,     | 1.77E-05| 6     | CYP3A4/CYP1A2/NOS3/HMOX1/NOS2/CYP1A           |
| GO:0005496   | steroid binding                                       | 1.88E-05| 5     | CYP3A4/ESR1/AR/NR3C2/PGR                     |
| GO:0004497   | monoxygenase activity                                  | 2.30E-05| 5     | CYP3A4/CYP1A2/NOS3/NOS2/CYP1A                |
| GO:0035173   | histone kinase activity                                | 2.56E-05| 3     | CDK1/CDK2/CHEK1                              |
| GO:0140297   | DNA-binding transcription factor binding               | 2.61E-05| 8     | PPARG/GSK3B/ESR1/MAPK14/JUN/STAT1/RXRA       |
| GO:0001091   | RNA polymerase II general transcription initiation     | 4.26E-05| 3     | ESR1/AR/AHR                                  |
| GO:0097110   | scaffold protein binding                               | 5.05E-05| 4     | KCNH2/NOS3/IKBKB/SCN5A                       |
| GO:0001223   | transcription coactivator binding                     | 6.57E-05| 3     | RELA/ESR1/AHR                                |
| GO:0042277   | peptide binding                                       | 6.73E-05| 7     | PPARG/INR/PK3CA/GSTM1/GSTP1/RXRA/HSP90AB1    |
| GO:0019903   | protein phosphatase binding                            | 0.000120241| 5     | AKT1/PPARG/MAPK14/STAT1/BCL2                  |
| GO:0097472   | cyclin-dependent protein kinase activity               | 0.000148077| 3     | CDK1/CDK2/CCNA2                              |
| GO:0051721   | protein phosphatase 2A binding                         | 0.000180016| 3     | AKT1/STAT1/BCL2                              |
| GO:0016712   | oxidoreductase activity, acting on paired donors,     | 0.000180016| 3     | CYP3A4/CYP1A2/CYP1A                          |
| GO:0033218   | amide binding                                         | 0.000215674| 7     | PPARG/INR/PK3CA/GSTM1/GSTP1/RXRA/HSP90AB1    |
| GO:0008395   | steroid hydroxylase activity                           | 0.000301701| 3     | CYP3A4/CYP1A2/CYP1A                          |
| GO:0016709   | oxidoreductase activity, acting on paired donors,     | 0.000326043| 3     | NOS3/NOS2/CYP1A                              |
| GO:0051879   | Hsp90 protein binding                                 | 0.000378447| 3     | AHR/CYP1A/AHSA1                              |
| GO:0035044   | Hsp70 protein binding                                 | 0.000406561| 3     | CDK1/BAX/CYP1A                               |
| GO:0140296   | general transcription initiation factor binding        | 0.000406561| 3     | ESR1/AR/AHR                                  |
Meaningful parameters were tested, such as network centralization (0.123), network heterogeneity (0.581), the average number of neighbors (4.136), and network density (0.071). The molecular function and target gene with the maximum degree were GO:0,004,674 (11) and ESR1 (8), respectively.

Biological processes in the top 5 terms were: response to the metal ion, response to lipopolysaccharide, response to molecules of bacterial origin, cellular response to chemical stress, and response to oxygen levels (Fig. 6A and Table 4). Moreover, from the topological properties of network construction (Fig. 6B), it could be known that the number of nodes was 65 and the number of edges was 256. Other meaningful parameters were further tested, such as network density (0.123), network centralization (0.131), the average number of neighbors (7.877), and network heterogeneity (0.602). The biological process and target gene with the maximum degree were GO:0,010,038 (15) and ICAM1 (16), respectively.

As shown in Fig. 7A and Table 5, membrane raft, membrane microdomain, membrane region, plasma membrane raft, and ion channel complex were the top 5 cellular components. Moreover, from the topological properties of network construction (Fig. 7B), it could be known that the number of nodes was 62 and the number of edges was 256. Other meaningful parameters were further tested, such as network density (0.123), network centralization (0.131), the average number of neighbors (7.877), and network heterogeneity (0.602). The biological process and target gene with the maximum degree were GO:0,004,674 (11) and ESR1 (8), respectively.

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**Fig. 5** The molecular function from GO enrichment analysis for target genes. The edges stand for the association between the nodes. In addition, GO enrichment in the top 30 from the molecular function had been obtained based on the threshold value of $p < 0.05$ (A). The network construction was shown as followed: the gene targets (green oval) and molecular functions (pink diamond) (B)

**Fig. 6** The biological process from GO enrichment analysis for target genes. The edges stand for the association between the nodes. In addition, GO enrichment in the top 30 from the molecular function had been obtained based on the threshold value of $p < 0.05$ (A). The network construction was shown as followed: the gene targets (green hexagon) and molecular functions (yellow rectangle) (B)
### Table 4 Biological process of GO analysis

| ID           | Biological process                                                                 | P value | Count | Gene name                                                                 |
|--------------|------------------------------------------------------------------------------------|---------|-------|---------------------------------------------------------------------------|
| GO:0000302   | response to reactive oxygen species                                                 | 4.66E-13| 13    | NOS3/AKT1/HMOX1/RELA/CASP3/MAPK8/JUN/CDK1/STAT1/BCL2/GSTP1/CDK2/CCNA2   |
| GO:0010038   | response to metal ion                                                               | 4.85E-13| 15    | CYP1A2/AKT1/HMOX1/CALM1/PPP3CA/MAPK8/JUN/CDK1/ICAM1/BCL2/VCAM1/CYP1A1/ICAM1/SCN5A |
| GO:0032496   | response to lipopolysaccharide                                                      | 2.35E-12| 14    | CYP1A2/NOS3/AKT1/NOS2/RELA/CASP3/MAPK14/MAPK8/JUN/ICAM1/VCAM1/GSTP1/CYP1A1/SLPI |
| GO:002237    | response to molecule of bacterial origin                                            | 3.96E-12| 14    | CYP1A2/NOS3/AKT1/NOS2/RELA/CASP3/MAPK14/MAPK8/JUN/ICAM1/GSTP1/CDK2/CCNA2 |
| GO:0062197   | cellular response to chemical stress                                                | 5.19E-12| 14    | NOS3/AKT1/PPARG/HMOX1/RELA/CASP3/MAPK8/JUN/CDK1/BCL2/GSTP1/CDK2/SLC2A4/CCNA2 |
| GO:0072593   | reactive oxygen species metabolic process                                          | 6.07E-12| 13    | CYP1A2/NOS3/AKT1/NOS2/INSR/MAPK14/XYD/F2/ICAM1/BCL2/GSTP1/CYP1A1/HSP90AB1 |
| GO:0009410   | response to xenobiotic stimulus                                                     | 8.61E-12| 13    | CYP3A4/CYP1A2/PPARG/RELA/PPP3CA/ICAM1/GSTM1/GSTP1/AHR/CYP1A1/NR1I2/CCNA2/HSP90AB1 |
| GO:0070482   | response to oxygen levels                                                           | 2.53E-11| 14    | AKT1/PPARG/HMOX1/NOS2/CASP3/ICAM1/BCL2/DPP4/VCAM1/CYP1A1/SLC2A4/PSMD3/CCNA2 |
| GO:0046677   | response to antibiotic                                                              | 3.55E-11| 13    | HMOX1/RELA/CASP3/JUN/CDK1/ICAM1/STAT1/BCL2/VCAM1/GSTP1/AR/CYP1A1/SLC2A4 |
| GO:0048608   | reproductive structure development                                                  | 8.29E-11| 14    | NOS3/AKT1/PPARG/INSR/CASP3/ESR1/MAPK14/BAX/ICAM1/AR/BCL2/PG/RXR/HSP90AB1 |
| GO:0061458   | reproductive system development                                                    | 9.09E-11| 14    | NOS3/AKT1/PPARG/INSR/CASP3/ESR1/MAPK14/BAX/ICAM1/AR/BCL2/PG/RXR/HSP90AB1 |
| GO:0001666   | response to hypoxia                                                                 | 1.13E-10| 13    | AKT1/HMOX1/NOS2/CASP3/ICAM1/BCL2/DPP4/VCAM1/CYP1A1/KNM1A/SLC2A4/PSMD3/CCNA2 |
| GO:0097191   | extrinsic apoptotic signaling pathway                                               | 1.43E-10| 11    | NOS3/AKT1/HMOX1/GSK3B/RELA/CASP3/BAX/ICAM1/AR/BCL2/GSTP1               |
| GO:0036293   | response to decreased oxygen levels                                                | 1.64E-10| 13    | AKT1/HMOX1/NOS2/CASP3/ICAM1/BCL2/DPP4/VCAM1/CYP1A1/KNM1A/SLC2A4/PSMD3/CCNA2 |
| GO:0048511   | rhythmic process                                                                   | 1.69E-10| 12    | NOS3/PPARG/NOS2/GSK3B/CASP3/ESR1/MAPK8/JUN/CDK1/AR/BCL2/PG/HAS2         |
| GO:0071466   | cellular response to xenobiotic stimulus                                            | 3.18E-10| 10    | CYP3A4/CYP1A2/ICAM1/GSTM1/GSTP1/AHR/CYP1A1/AR/CCNA2/SLC2A4/PSMD3/CCNA2 |
| GO:0034612   | response to tumor necrosis factor                                                   | 3.22E-10| 12    | AKT1/RELA/CASP3/MAPK14/ICAM1/STAT1/IKBKB/VCAM1/GSTP1/SLC2A4/PSMD3/HAS2 |
| GO:0008585   | female gonad development                                                            | 8.55E-10| 8     | NOS3/INSR/CASP3/ESR1/BAX/ICAM1/BCL2/PG/RXR/HSP90AB1                     |
| GO:0051090   | regulation of DNA-binding transcription factor activity                             | 1.09E-09| 13    | AKT1/PPARG/HMOX1/RELA/PPP3CA/ESR1/MAPK14/MAPK8/JUN/ICAM1/AR/IKBKB/PIM1 |
| GO:0048732   | gland development                                                                  | 1.15E-09| 13    | AKT1/HMOX1/INSR/RELA/ESR1/JUN/XYD/BAX/AR/BCL2/CYP1A1/PG/RXR/HAS2        |
| GO:0046545   | development of primary female sexual characteristics                                | 1.29E-09| 8     | NOS3/INSR/CASP3/ESR1/BAX/ICAM1/BCL2/PG/RXR/HAS2                       |
| GO:0065359   | positive regulation of reactive oxygen species metabolic process                    | 1.39E-09| 8     | AKT1/INSR/MAPK14/XYD/F2/ICAM1/GSTP1/AR/IKBKB/VCAM1/GSTP1/SLC2A4/PSMD3/CCNA2 |
| GO:0021237   | negative regulation of extrinsic apoptotic signaling pathway                        | 1.63E-09| 8     | NOS3/AKT1/HMOX1/RELA/ICAM1/AR/BCL2/GSTP1                               |
| GO:0006979   | response to oxidative stress                                                        | 1.84E-09| 13    | NOS3/AKT1/HMOX1/RELA/CASP3/MAPK8/JUN/CDK1/STAT1/BCL2/GSTP1/CDK2/CCNA2 |
| GO:0071356   | cellular response to tumor necrosis factor                                          | 2.29E-09| 11    | AKT1/RELA/MAPK14/ICAM1/STAT1/IKBKB/VCAM1/GSTP1/SLC2A4/PSMD3/CCNA2     |
of nodes was 57 and the number of edges was 131. Other meaningful parameters were further tested, such as network density (0.082), network centralization (0.174), the average number of neighbors (4.596), and network heterogeneity (0.690). The cellular components and target genes with a maximum degree were GO:0051098 (12) and SCN5A (10), respectively. The results showed that the pharmacological effects of bioactive ingredients were closely linked to MAPK-coupled receptor activity or signal pathways. In addition, transcription factors have a significant impact on signal transduction.

**KEGG enrichment analysis for target genes and mapping**

As shown in Table 6 and Fig. 8A, there were 61 overlapping genes primarily enriched in 20 signaling transduction pathways. The top 5 (generatio) signaling pathways were fluid shear stress and atherosclerosis, Kaposi sarcoma-associated herpesvirus infection, Epstein-Barr virus infection, AGE-RAGE signaling pathway in diabetic complications, and human immunodeficiency virus 1 infection. In addition, Topological properties of network (Fig. 8B) demonstrated that the figure of edges and nodes respectively was 218 and 57. Other parameters including network density (0.137), network centralization (0.210), the average number of neighbors (7.649), and network heterogeneity (0.691) were also analyzed. The signaling pathway and target protein with a maximum degree were hsa05418 (15) and RELA (19), respectively. The top 3 mapping signaling pathways were fluid shear stress and atherosclerosis (Fig. 9), Kaposi sarcoma-associated herpesvirus infection (Fig. 10) and Epstein-Barr virus infection (Fig. 11), indicating that these signaling pathways play an important role in the pharmacological effect of Acoritataninowii Rhizoma on AD.
Molecular docking between ingredients and target proteins

Binding interactions of the compounds (MOL000422, MOL003542, MOL003576, MOL003578) and collecting the native ligands (AKT1, CASP3, ESR1 and JUN, MAPK14) with target proteins were shown in Table 7. The binding energy range of each target proteins were as followed: AKT1 (-9.07 to -6.04 kcal/mol), CASP3 (-7.9 to -6.14 kcal/mol), ESR1 (-10.85 to -5.65 kcal/mol), JUN (-8.98 to -6.31) and MAPK14 (-9.29 to -6.63 kcal/mol). Additionally, the highest binding affinity of each biologically active ingredient were as followed: MOL00042 (ESR1: -6.92 kcal/mol), MOL003542 (CASP3: -6.96 kcal/mol), MOL003576 (CASP3: -7.9 kcal/mol), and MOL003578 (ESR1: -10.85 kcal/mol). The docking diagrams and binding energy were depicted as
| ID     | Pathway                                              | P value   | Count | Gene name                                                                 |
|--------|------------------------------------------------------|-----------|-------|---------------------------------------------------------------------------|
| hsa05418 | Fluid shear stress and atherosclerosis               | 1.43E-14  | 15    | NOS3/AKT1/HMOX1/RELA/CALM1/MAPK14/MAPK8/JUN/ICAM1/GSTM1/BCL2/IKBKB/VCAM1/GSTP1/HSP90AB1 |
| hsa04933 | AGE-RAGE signaling pathway in diabetic complications | 8.81E-14  | 13    | NOS3/AKT1/RELA/CASP3/MAPK14/MAPK8/JUN/BAX/ICAM1/STAT1/BCL2/VCAM1/PPM1    |
| hsa05167 | Kaposi sarcoma-associated herpesvirus infection      | 2.98E-11  | 14    | AKT1/GSK3B/RELA/CASP3/CALM1/PPP3CA/MAPK14/MAPK8/JUN/BAX/ICAM1/STAT1/IKBKB/PI3KCG |
| hsa05169 | Epstein-Barr virus infection                         | 5.51E-11  | 14    | AKT1/RELA/CASP3/MAPK14/MAPK8/JUN/BAX/ICAM1/STAT1/BCL2/IKBKB/CDK2/P53D3/CCNA2 |
| hsa05161 | Hepatitis B                                          | 7.35E-10  | 12    | AKT1/RELA/CASP3/MAPK14/MAPK8/JUN/BAX/STAT1/BCL2/IKBKB/CDK2/CCNA2         |
| hsa05170 | Human immunodeficiency virus 1 infection             | 1.36E-09  | 13    | AKT1/RELA/CASP3/CALM1/PPP3CA/MAPK14/MAPK8/JUN/CDK1/BAX/BCL2/IKBKB/HEK1   |
| hsa05162 | Measles                                              | 2.03E-09  | 11    | AKT1/GSK3B/RELA/CASP3/MAPK8/JUN/BAX/STAT1/BCL2/IKBKB/CDK2               |
| hsa04659 | Th17 cell differentiation                            | 2.33E-09  | 10    | RELA/PPP3CA/MAPK14/MAPK8/JUN/STAT1/IKBKB/AHR/RXRA/HSP90AB1               |
| hsa05145 | Toxoplasmosis                                        | 3.66E-09  | 10    | AKT1/NOS2/RELA/CASP3/MAPK14/MAPK8/STAT1/BCL2/IKBKB/PI3KCG               |
| hsa04722 | Neurotrophin signaling pathway                       | 6.63E-09  | 10    | AKT1/GSK3B/RELA/CALM1/MAPK14/MAPK8/JUN/BAX/BCL2/IKBKB                   |
| hsa05222 | Small cell lung cancer                                | 1.06E-08  | 9     | AKT1/NOS2/RELA/CASP3/BAX/BCL2/IKBKB/CDK2/RXRA                            |
| hsa04657 | IL-17 signaling pathway                               | 1.28E-08  | 9     | GSK3B/RELA/CASP3/MAPK14/MAPK8/JUN/MMP1/IKBKB/HSP90AB1                   |
| hsa05215 | Prostate cancer                                       | 1.70E-08  | 9     | AKT1/GSK3B/RELA/AR/BCL2/IKBKB/GSTP1/CDK2/HSP90AB1                       |
| hsa04914 | Progesterone-mediated oocyte maturation               | 2.22E-08  | 9     | AKT1/MAPK14/MAPK8/CDK1/PKRACA/CDK2/PGR/CCNA2/HSP90AB1                   |
| hsa05166 | Human T-cell leukemia virus 1 infection               | 2.28E-08  | 12    | AKT1/RELA/PPP3CA/MAPK8/JUN/BAX/ICAM1/IKBKB/PKRACA/CDK2/HEK1/CCNA2        |
| hsa05152 | Tuberculosis                                         | 3.09E-08  | 11    | AKT1/NOS2/RELA/CASP3/CALM1/PPP3CA/MAPK14/MAPK8/BAX/STAT1/BCL2           |
| hsa04625 | C-type lectin receptor signaling pathway             | 3.15E-08  | 9     | AKT1/RELA/CALM1/PPP3CA/MAPK14/MAPK8/JUN/STAT1/IKBKB                 |
| hsa04931 | Insulin resistance                                   | 4.39E-08  | 9     | NOS3/AKT1/INSR/GSK3B/RELA/MAPK8/IKBKB/SLC2A4/PYGM                      |
| hsa04668 | TNF signaling pathway                                 | 6.03E-08  | 9     | AKT1/RELA/CASP3/MAPK14/MAPK8/JUN/ICAM1/IKBKB/VCAM1                   |
| hsa04932 | Non-alcoholic fatty liver disease                    | 6.20E-08  | 10    | AKT1/INSR/GSK3B/RELA/CASP3/MAPK8/JUN/BAX/IKBKB/RXRA                     |
| hsa04380 | Osteoclast differentiation                            | 1.92E-07  | 9     | AKT1/PPARG/RELA/PPP3CA/MAPK14/MAPK8/JUN/STAT1/IKBKB                 |
| hsa04926 | Relaxin signaling pathway                             | 2.06E-07  | 9     | NOS3/AKT1/NOS2/RELA/MAPK14/MAPK8/JUN/MMP1/PKRACA                     |
| hsa01522 | Endocrine resistance                                 | 3.10E-07  | 8     | AKT1/ESR1/MAPK14/MAPK8/JUN/BAX/BCL2/PKRACA                           |
| hsa04910 | Insulin signaling pathway                             | 3.45E-07  | 9     | AKT1/INSR/GSK3B/CALM1/MAPK8/IKBKB/PKRACA/SLC2A4/PYGM                  |
| hsa04915 | Estrogen signaling pathway                            | 3.67E-07  | 9     | NOS3/AKT1/CALM1/ESR1/JUN/BCL2/PKRACA/PHR/HSP90AB1                     |
| hsa04917 | Prolactin signaling pathway                           | 4.48E-07  | 7     | AKT1/GSK3B/RELA/ESR1/MAPK14/MAPK8/STAT1                             |
followed (details of Fig. 12–18 are given in the supplemental material):

1. interactions with CASP3: MOL000422 and CASP3 (-6.14 kcal·mol⁻¹) (Fig. 13A), MOL003542 and CASP3 (-6.96 kcal·mol⁻¹) (Fig. 13B), MOL003576 and CASP3 (-7.9 kcal·mol⁻¹) (Fig. 13C), MOL003578 and CASP3 (-7.57 kcal·mol⁻¹) (Fig. 13D);

2. interactions with ESR1: MOL000422 and ESR1 (-6.92 kcal·mol⁻¹) (Fig. 14A), MOL003542 and ESR1 (-5.65 kcal·mol⁻¹) (Fig. 14B), MOL003576 and ESR1 (-7.22 kcal·mol⁻¹) (Fig. 14C), MOL003578 and ESR1 (-10.85 kcal·mol⁻¹) (Fig. 14D);

3. interactions with JUN: MOL000422 and JUN (-6.45 kcal·mol⁻¹) (Fig. 15A), MOL003542 and JUN (-6.31 kcal·mol⁻¹) (Fig. 15B), MOL003576 and JUN (-7.33 kcal·mol⁻¹) (Fig. 15C), MOL003578 and JUN (-8.98 kcal·mol⁻¹) (Fig. 15D);

4. interactions with MAPK14: MOL000422 and MAPK14 (-6.63 kcal·mol⁻¹) (Fig. 16A), MOL003542 and MAPK14 (-7.1 kcal·mol⁻¹) (Fig. 16B), MOL003576 and MAPK14 (-6.81 kcal·mol⁻¹) (Fig. 16C), MOL003578 and MAPK14 (-9.29 kcal·mol⁻¹) (Fig. 16D);

5. interactions with AKT1: MOL000422 and AKT1 (-6.48 kcal·mol⁻¹) (Fig. 17A), MOL003542 and AKT1 (-6.04 kcal·mol⁻¹) (Fig. 17B), MOL003576 and AKT1 (-6.4 kcal·mol⁻¹) (Fig. 17C), MOL003578 and AKT1 (-9.07 kcal·mol⁻¹) (Fig. 17D).

The results showed that these bioactive ingredients and core target proteins were of great significance to the possible mechanism of Acoritataninowii Rhizoma defense against AD.

Discussion

Acoritataninowii Rhizoma was widely used in Alzheimer's disease, depression, and other diseases, but its mechanism on AD has not been fully elucidated (Li et al. (2019)).
The relationship between drugs and diseases were explored by the principle of network theory and systems biology combined with molecular docking. Furthermore, Acoritaninowii Rhizoma treated Alzheimer’s disease through multiple components and target genes, providing a relevant theoretical basis for the rational use of drugs and the development of new drugs (Huang et al. 2014). Details of 4 active ingredients and 81 target genes from Acoritaninowii Rhizoma were obtained from TCMSP, TCMID, and other databases. In the Database of DrugBank, GeneCards, and other databases, a total of 6765 target genes were found to be AD-specific target genes. In the intersection of potential targets, 61 overlapping genes were calculated, accounting for 0.9% of the total genes. Through PPI network analysis, AKT1, AR, CASP3, ESR1, JUN, MAPK8, and other proteins may be the core target genes involved in the pathology of Alzheimer's disease. GO enrichment analysis showed that overlapping genes may be involved in protein serine/threonine kinase activity, ubiquitin-like protein ligase binding, RNA polymerase II-specific DNA-binding transcription factor binding, ubiquitin-protein ligase binding. KEGG enrichment analysis showed that overlapping genes were associated with fluid shear stress and atherosclerosis, the AGE-RAGE signaling pathway in diabetic complications, and human immunodeficiency virus 1 infection.

Additionally, the molecular docking results showed that Kaempferol and Cycloartenol were important bioactive constituents, and AKT1 and ESR1 are promising target genes.

**Active ingredients**

The 4 active ingredients screened by ADME and other conditions were: Cycloartenol, (1R, 3aS, 4R, 6aS) -1, 4-bis (3,4-dimethoxyphenyl)—1, 3, 3a,
4,6,6a-hexahydrofuro[4,3-c]furan, 8-Isopentenyl-kaempferol, kaempferol.

Kaempferol has an effective effect of inhibiting inflammatory cell function and inhibiting the expression of pro-inflammatory cytokines and chemokines, and its pharmacological activity can prevent or treat neurodegenerative diseases (Calderon-Montano et al. 2011; Devi et al. 2015).

The use of kaempferol in a model of Alzheimer's disease in flies reduced neurotoxic exercise and cognitive impairment (Beg et al. 2018), protecting PC12 and T47D cells from amyloid-induced toxic β. In addition, the use of kaempferol leads to a decrease in markers of oxidative stress, delay climbing ability, and memory loss.

Cycloatonol is a plant sterol compound with activities including antioxidants, anti-Alzheimer’s disease (Zhang et al. 2017). Cycloartenol has non-competitive inhibitory activity against acetylcholinesterase (AChE) and also has inhibitory activity against butyrylcholinesterase (BChE). Cycloartenol as an AChE inhibitor can exert its anti-Alzheimer’s effects (Jung et al. 2010).

**PPI**

By using PPI network analysis, a sum of 59 target proteins are deem to be highly core target proteins, including AKT1, AR, CASP3, ESR1,JUN, MAPK8, MAPK14,NOS3,RELA, etc.

The AKT signaling pathway might be the pathological biochemical basis for a sharp decrease in glucose/energy metabolism in the Alzheimer’s disease brain (Hoyer (1998)). CASP3 might be a specific apoptosis mediator and performer(TCM) (Mao et al. 2008). AR (androgen...
receptor) plays an important role in the pathogenesis of castration-resistant prostate cancer, involving gene amplification and mutation, regulator changes, apoptosis resistance, etc. (Chandrasekar et al. 2015). MAPK8 can significantly upregulate the expression of STAT1 and AKT1 and downregulate the expression levels of the PTGS2 and JUN genes (Lui et al. 2019). AKT1 protein is a protein associated with Alzheimer's pathology that promote therapeutic strategies like synaptic Akt1-mTOR signaling which may provide new targets for disease-remission therapy in AD (Ahmad et al. 2017).

In studies of Alzheimer's risk in patients with type 2 diabetes, activation of AKT1 favors ab protein upstream and normal phosphorylation with Tau protein. AKT1 rs2498786
in the insulin signaling pathway may be associated with AD risk, and different genotypes may affect AK1 expression levels. Genetic variation in AKT1 rs2498786 may lead to AD occurrence (Liu et al. 2015).

Apoptosis levels as well as apoptosis factors are reflected by caspase-3. Caspase-3 plays a crucial role in apoptosis and is a common apoptosis signaling pathway (Abdel Shakor et al. 2015, Edgington-Mitchell and Bogyo 2016, Morales-Cano et al. 2013).

**GO**

On the basis of GO functional analysis, and the basis of molecular function, biological processes, and cell composition criteria, the participation of common target genes in more than 30 signal transduction functions were evaluated, such as lipopolysaccharides, metal ions; MAPK-associated protein serine/threonine kinases, etc. These GO enrichments have important implications for the anti-Alzheimer’s effects of Acoritataninowii Rhizoma. Function/process is associated with synapses or postsynaptic membranes in cells with the following target genes: AKT1, BCL2, CASP3, CDK1, EAR1, JUN, PPARG.

Mitogen-activated protein kinase 14 (p38MAPK) is activated by cellular oxidative stress when DNA damage occurs, it plays an important role in the pathology of Alzheimer’s disease and is a potential therapeutic target for cognitive dysfunction of the neuroinflammatory-synaptic dysfunction cycle (de Oliveira et al. 2015). Controlling p38a MAPK in a mouse model of Alzheimer’s disease can alleviate the associated EC dysfunction caused by AD (Rutigliano et al. 2018). MiR-22-3p can reduce Aβ deposition and alleviate AD symptoms by modulating the expression of MAPK14 (Ji et al. 2019). Additionally, down-regulating the phosphorylation level of MAPK14 can reduce tau protein phosphorylation (Yu et al. 2016).

| Ingredients | Structure | Binding Energy/(kcal•mol⁻¹) |
|-------------|-----------|---------------------------|
| kaempferol  | ![Structure](image1) | -6.4                      |
| 8-Isopentenyl-kaempferol | ![Structure](image2) | -6.0                      |
| (1R,3aS,4R,6aS)-1,4-bis(3,4-dimethoxyphenyl)-1,3,3a,4,6,6a-hexahydrofuro[4,3-c]furan | ![Structure](image3) | -6.4                      |
| Cycloartenol | ![Structure](image4) | -9.0                      |
MW150 can selectively modulate the neuroinflammatory response associated with pathological progression (Zhou et al. 2017).

AP-1 (activating protein-1) is a general term that refers to dimer transcription factors composed of Jun, Fos, or ATF (activated transcription factor), the subunits can bind to a common DNA site (AP-1 binding site) (Karin et al. 1997). AP-1 is able to block the activation of nuclear factors (NF)-kB and mitoticogen-activated protein kinase (MAPK), inducing prolonged microglial cell activation, may downregulate neuroprotective events in the neurodegenerative disease brain (Youssef et al. 2019).

In addition, NMB-induced COX-2 and IL-6 expression are mediated by p65 and c-Jun activation (Zhu et al. 2019).

Therefore, MAPK may play an important role in three biological functions.
**Fig. 13** The 3D molecular models between CASP3 and drug/component as followed: A MOL000422-CASP3, B MOL003542-CASP3, C MOL003576-CASP3, D MOL003578-CASP3

**Fig. 14** The 3D molecular models between ESR1 and drug/component as followed: A MOL000422-ESR1, B MOL003542-ESR1, C MOL003576-ESR1, D MOL003578-ESR1
Fig. 15 The 3D molecular models between JUN and drug/component as followed: A MOL000422- JUN, B MOL003542- JUN, C MOL003576- JUN, D MOL003578- JUN

Fig. 16 The 3D molecular models between MAPK14 and drug/component as followed: A MOL000422- MAPK14, B MOL003542- MAPK14, C MOL003576- MAPK14, D MOL003578- MAPK14
In addition, the possible pathways of Acoritataninowii Rhizoma to AD were analyzed through KEGG enrichment. The pharmacological effect of Acoritataninowii Rhizoma on AD involves multiple signaling pathways such as fluid shear stress and atherosclerosis, Kaposi sarcoma-associated herpesvirus infection, Epstein-Barr virus infection, AGE-RAGE signaling pathway in diabetes complications, human immunodeficiency virus 1 infection, hepatitis B, etc.

Fluid shear stress of the signaling pathway and the induction of atherosclerosis in endothelial cells Fractalkine increased the expression of mRNA, resulting in the arteriosclerosis of plaques, which caused cardiovascular and cerebrovascular diseases in turn (Ruze et al. 2018).

Glycosylation end products (AGEs) and their receptor RAGE rank fourth in this article, and RAGE and its bait receptor soluble RAGE may prevent AD by influencing the transport of β-amyloid to the brain or by manipulating inflammatory mechanisms of Pathogenesis (Srikanth et al. 2011).

Activating the RAGE receptor, and the transcription factor NF-κB, can cause inflammation and oxidative stress, destroy the structure and function of brain neurons and the hippocampus, and lead to a decline in learning and memory function (Salminen et al. 2009).

TNF-α (tumor necrosis factor-α) is a pro-inflammatory cytokine that participates in cell differentiation and induces apoptosis or inflammation by upregulating signaling pathways such as MAPK and NF-κB (Chengzong (2019)).

The PI3K/AKT signaling pathway can exert anti-AD effects in traditional Chinese medicine, which has been confirmed in a large number of studies. The signaling pathway consisting of PI3K and AKT is an anti-apoptotic and pro-survival signaling pathway, it has been shown to play a key role in neuroprotection and inhibition of apoptosis by enhancing soD expression, as it is associated with high phosphorylation of the tau protein (Liang et al. 2020; Matsuda et al. 2018, Liu et al., (2019), Sun et al. (2018)).

**Molecular docking**

This study also proposes to use a wide range of research methods such as network pharmacology and molecular docking to verify protein–ligand interactions, supplementing the pharmacological effect of Acoritataninowii Rhizoma on AD. Based on their high degree of representation in the Acoritataninowii module-component-target-AD network, multiple target genes (ESR1, CASP3, JUN, MPK14, and AKT1) and bioactive ingredients (MOL000422, MOL003542, MOL003576, and MOL003578) were selected for molecular docking. The molecular docking
Fig. 18 The results of 2D interaction of molecular docking
results are similar to network analysis, and these bioactive constituents and core target genes may be potential keys to the pharmacological effect of Acoritataninowii Rhizoma on AD. AKT1 and CASP3 exhibited a high affinity for these active ingredients. Combined with the results of the analysis of network pharmacological studies, it seems that the pharmacological effects of the bioactive constituents (MOL000422 and MOL003578) derived from Acoritataninowii Rhizoma on AD are related to AKT1 and ESR1, respectively.

This study, however, had some limitations. Additionally, there are some unknown targets and compounds. Due to this, it is not possible to fully reveal the pharmacological effects of herbal formulas. Moreover, the incompleteness of the database and the targets from different databases may lead to biased results based on data mining methods. In further research, metabolomics analyses and animal experiments will be used to confirm predictions made by docking and network pharmacology studies. Additionally, using liquid chromatography-mass spectrometry (LC–MS) and
metabolomics (pathways) to identify core metabolites and associated signaling pathways associated with the anti-AD activity of Acoritataninowii Rhizoma.

Conclusion

All in all, the pathogenesis of AD is unclear. The signal transduction are cross-linked and mutually modulated, presenting a multi-target and multi-pathway pattern.

The processes such as the protein serine/threonine kinase associated with MAPK and the signaling pathways such as fluid shear stress and atherosclerosis, AGE-RAGE were regulated and affected by Acoritataninowii Rhizoma respectively. The Cycloartenol and kaempferol collected from Acoritataninowii Rhizoma are related to ESR1 and AKT1 respectively, which may be the core target genes for treating AD.

The result indicated the multi-compound-multi-target-multipathway action mode of TCM. The pharmacological effects of Acoritataninowii Rhizoma on AD and the targets and signaling pathways with the compounds need further analysis and validation.

Author contribution All authors contributed to the study conception and design. Jia-Li Pang, Bai-xian Zhou and Zhi-Kun Qiu designed the experimental methods. Han-Biao Wu, Wei-Qiang Zeng, Bai-xian Zhou and Zhi-Kun Qiu, Fan Yang collected and analyzed the data. Bai-xian Zhou and Zhi-Kun Qiu, Fan Yang wrote the initial draft of the manuscript. All authors read and approved the final manuscript.

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Data availability 1. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

2. All data generated or analyzed during this study are included in this published article.

Declarations

Consent to publish All the authors listed have approved the manuscript that is enclosed.

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

Consent to participate Not applicable.

Conflict of Interest The authors declare no conflict of interest.

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