

## Research Article

# Mechanisms of Intervertebral Disc Degeneration Treatment with Deer Antlers Based on Network Pharmacology and Molecular Docking

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**Background.** With the aging of the population, the prevalence of IVDD increases preoperatively. How to better treat IVDD has become an important clinical issue. Deer antlers proved to have a great effect on the treatment of IVDD in many studies, but the molecular mechanism has not been clarified. **Objective.** To investigate the molecular mechanism and target of deer antlers in the treatment of IVDD. **Methods.** Compounds from deer antlers were collected and targets were predicted using HERB, TCMSP, TCMID, SwissADME, and SwissTargetPrediction. Collection of disease targets for IVDD was done using GeneCards, TTD, DrugBank, DisGeNET, and OMIM. Cytoscape 3.7.2, AutoDock Vina (v1.1.2), and R software were used for data analysis and the construction of network diagrams. **Results.** A total of 5 active compounds from deer antlers were screened and 104 therapeutic targets were predicted. A total of 1023 IVDD disease targets were collected. Subsequently, PPI network prediction analysis was performed for disease and treatment targets, and 112 core targets were collected after screening. After obtaining the core target, we used the clusterProfiler software package of R software to carry out GO and KEGG enrichment analyses for the core target and plot the bubble maps. According to the GO enrichment results, the main biological processes of IVDD treatment by deer antlers lie in the rhythmic process, mRNA catabolic process, and G1/S transition of the mitotic cell cycle. KEGG results were mainly related to the PI3K-Akt signaling pathway, thyroid hormone signaling pathway, and Notch signaling pathway. Molecular docking results showed that estrone had the best docking results on ESRI. **Conclusion.** Deer antlers are rich in various compounds that can prevent the development of IVDD by upregulating the PI3K-Akt signaling pathway and Notch signaling pathway. Its key compounds estradiol and estrone can reduce the inflammatory response and oxidative stress in tissues and organs, thus slowing down the progression of IVDD. Estrone, the active compound in deer antlers, was found by molecular docking to have good results against ESRI, the target of the disease, which may be a potential site for drug therapy.

## 1. Introduction

Deer antlers (*Cervi Cornu Pantotrichum*) refer to the outgrowths on the forehead of the male of sika deer and red deer, which are covered with a layer of velvety fur [1]. Deer antlers, owning a beneficial effect of tonifying “kidney-yang,” are normally used to treat low back pain, soreness of the knee, morbid vaginal discharge, and so on [2]. The efficiency of deer antlers is dependent on their bioactive

components. Recent studies reported that the deer antlers’ significant ingredients are polysaccharides, polypeptides, and free amino acids [3]. The nucleus pulposus, cartilage endplates, and annulus fibrosus constitute the intervertebral disc, which can keep the spine stable and flexible [4]. One of the main factors contributing to low back pain is intervertebral disc degeneration (IVDD) [5]. With the aging of the population, the prevalence of IVDD is increasing in proportion [6]. One study showed that the incidence of

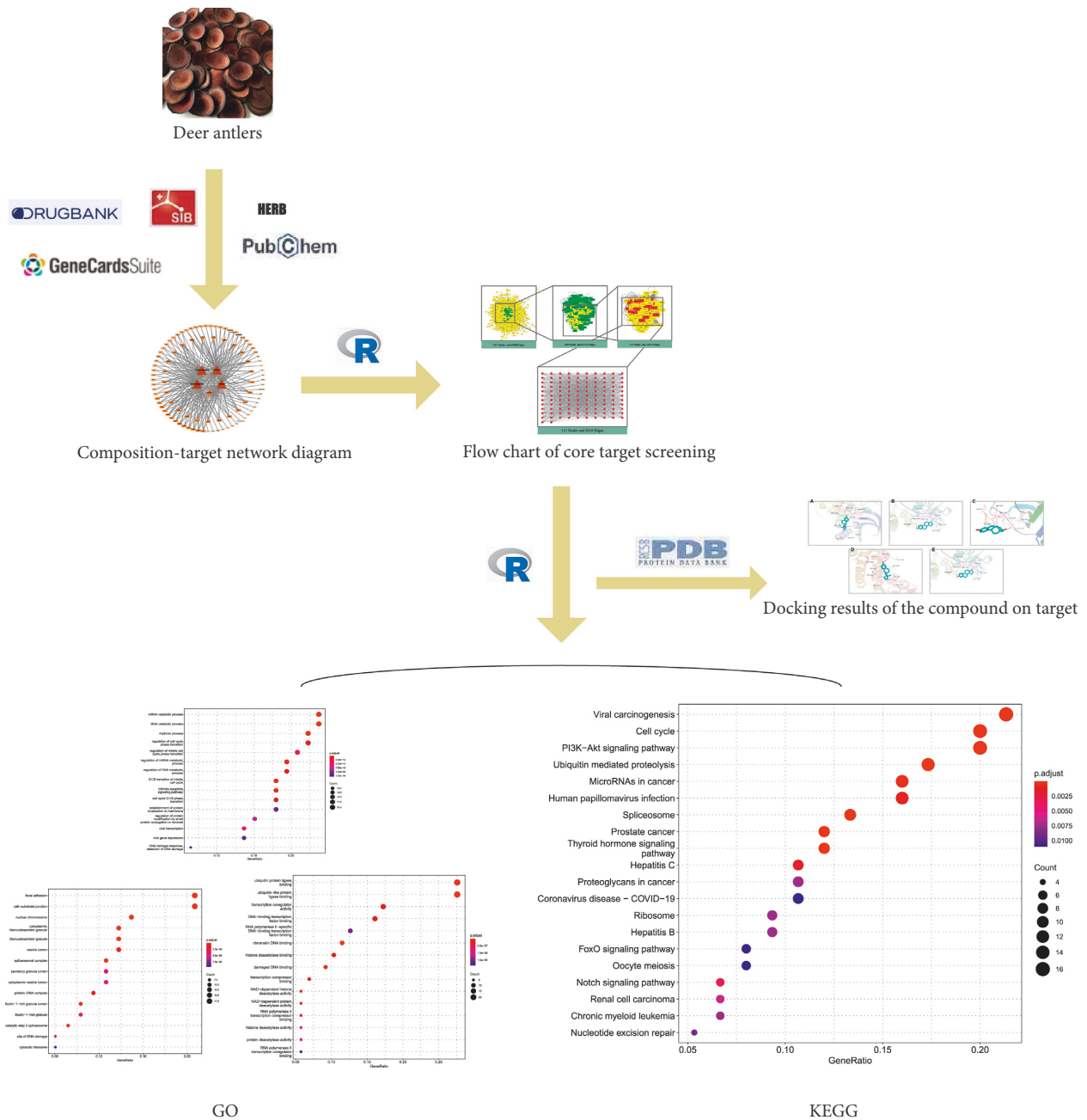


FIGURE 1: Brief flowchart with network pharmacology and molecular docking.

TABLE 1: Active ingredients of TCM.

Ingredient ID	Ingredient name
HBIN001991	17-Beta-estradiol
HBIN015508	Alpha-estradiol
HBIN025818	Estragole
HBIN025821	Estrone
HBIN037857	Estrone

IVDD in the whole lumbar spine was 31.6% in men and 44.7% in women [7]. People have thought that inflammation and oxidative stress are closely related to IVDD. Polysaccharides have good antioxidant properties [8]. Furthermore, deer antler peptide, exhibiting significant anti-inflammatory

and antioxidative effects, can effectively protect osteoblasts [9–11]. Meanwhile, proteins contained in deer antlers protect against oxidative stress and inflammation [3, 12].

Network pharmacology, which is based on the concept of a multilevel and multiangle interaction network among diseases, genes, targets, and drugs, can observe the interventional mechanism and influence of drugs on the disease network systematically and comprehensively [13]. It can generate complex networks of interactions according to target molecules, biological functions, and bioactive compounds to clarify the mechanism of action of TCM prescriptions at the molecular level [14]. Up to now, network pharmacology has been used in many studies of Chinese

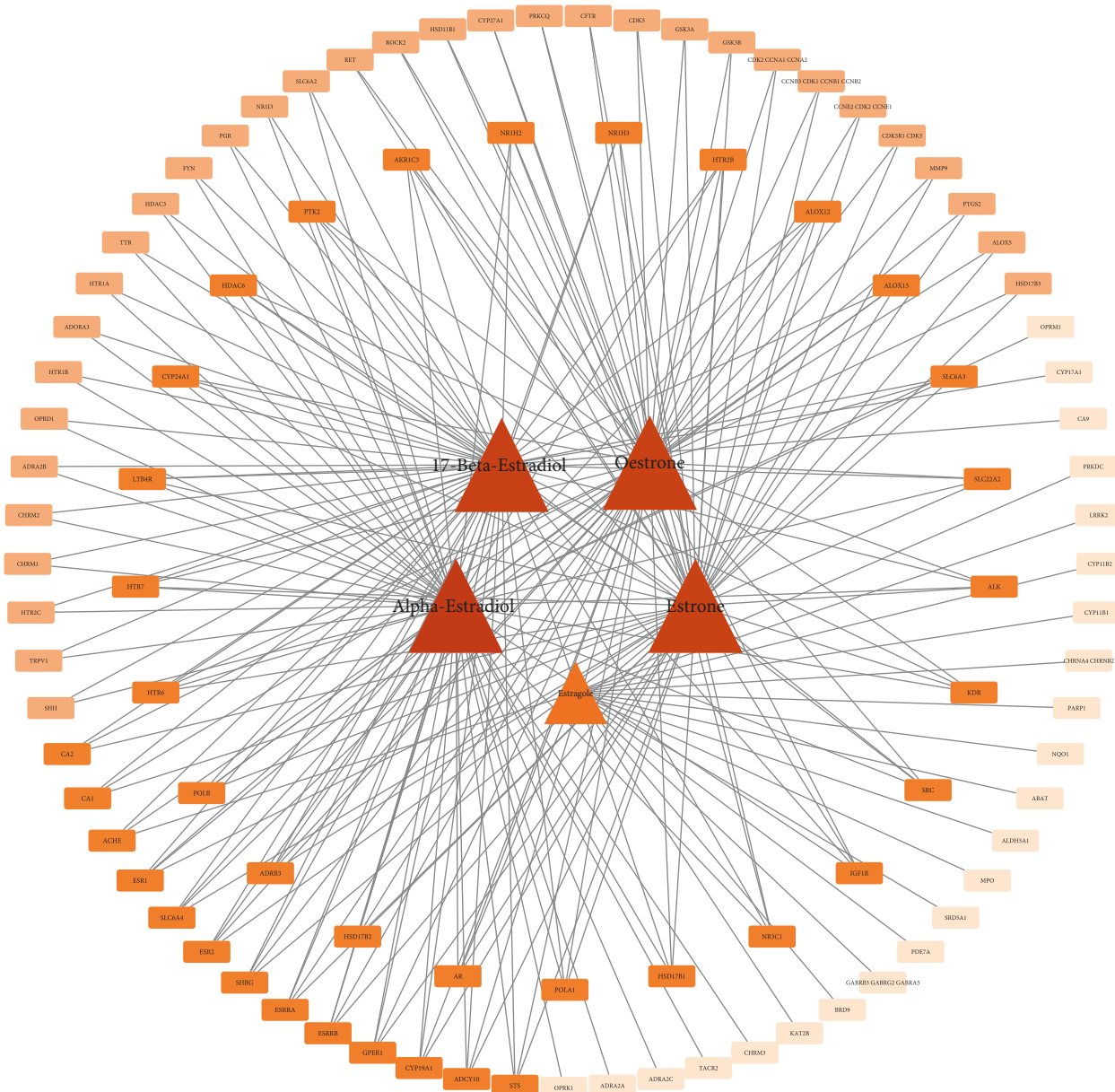


FIGURE 2: Composition-target network diagram. The triangle represents the active compound and the rectangle represents the target site of action.

TABLE 2: Key targets of TCM.

Compound	Degree
Alpha-estradiol	58
17-Beta-estradiol	55
Estrone	54
Estrone	54
Estragole	17

herbal medicine and its preparations [15]. Molecular docking is an effective tool in structural molecular biology and computer-assisted drug design that predicts a ligand's major binding mode(s) with a target protein [16]. Due to the unique therapeutic properties, deer antlers have attracted much research interest. So far, nevertheless, the target and mechanism of deer antlers in the treatment of IVDD have

not been elucidated in the literature, and people know little about it, which limits the further development and application of deer antlers to some extent. This study aims to explore the potential mechanism of deer antlers action from the perspective of network pharmacology.

## 2. Materials and Methods

**2.1. Component-Target Network.** We searched for active ingredients in deer antlers using HERB (<https://herb.ac.cn/>), TCMSP (<https://tcm-sp-e.com/>), TCMID (<https://47.100.169.139/tcmid/>), and other Chinese medicine database platforms as well as the literature. Immediately afterward, the collected active ingredients were screened for ADME on the SwissADME (<https://www.swissadme.ch/index.php>) using the following criteria: "GI absorption" as "HIGH" and "YES" for

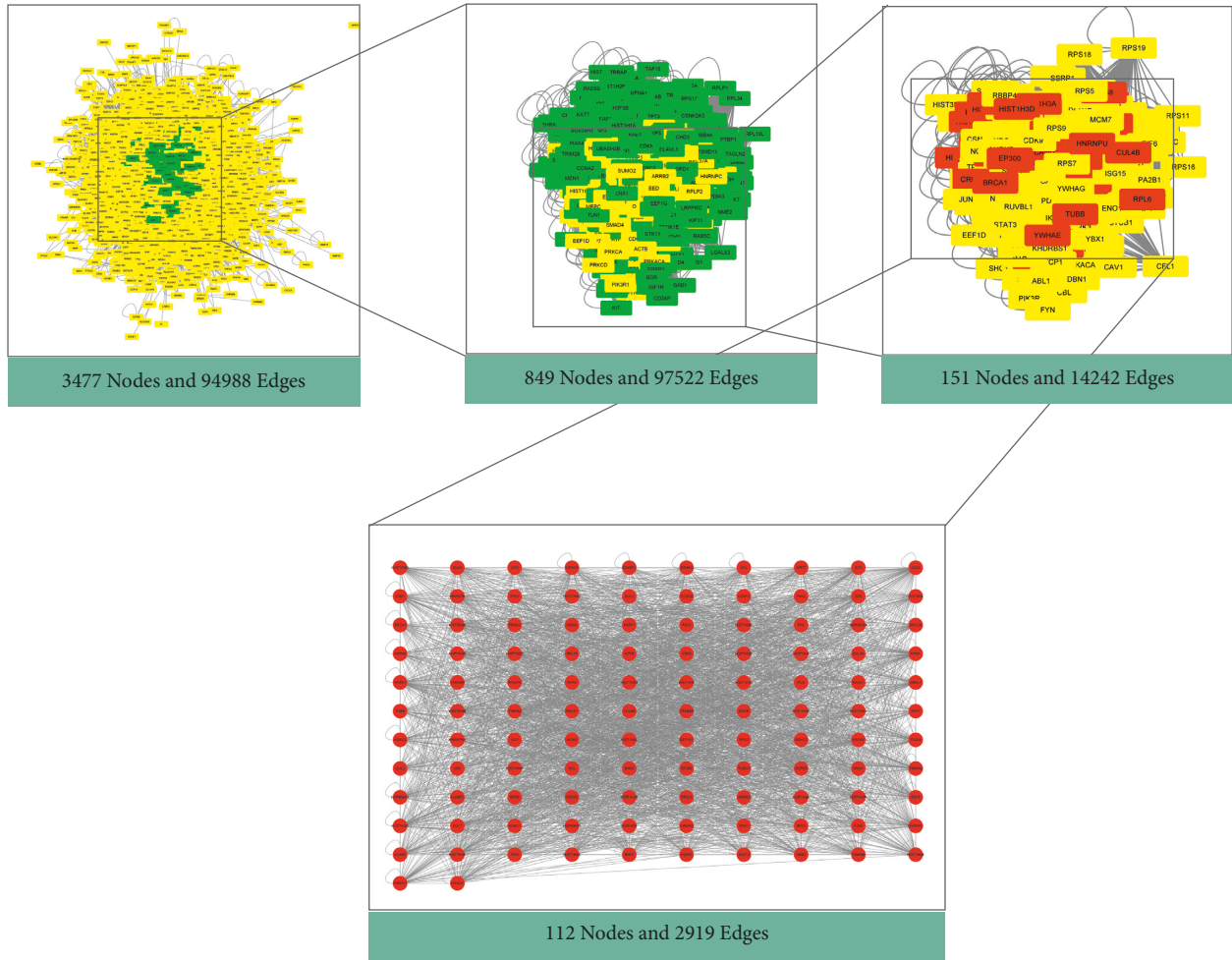


FIGURE 3: Flowchart of core target screening.

TABLE 3: 112 hub genes.

Gene name	Gene name	Gene name	Gene name	Gene name	Gene name	Gene name
NTRK1	SIRT7	HNRNPU	MDM2	YWHAG	TUBB	HIST1H4A
ESR1	YWHAZ	HUWE1	YWHAQ	ARRB2	HNRNPK	HIST4H4
CDK2	CAND1	HDAC5	VHL	FUS	NCL	HIST2H4B
CUL3	OBSL1	HNRNPA1	HSPA5	STAU1	ILF3	HIST1H4I
TP53	NPM1	RPA1	RPA2	HDAC2	RPS3	RPS2
MCM2	ITGA4	HIST1H3C	HSPA8	HDAC3	EIF4A3	CUL4A
EGFR	EP300	HIST1H3E	EEF1A1	TARDBP	HIST1H4C	HNRNPM
XPO1	HSP90AB1	HIST1H3I	CREBBP	RPS27A	HIST1H4H	SMARCA4
FN1	HDAC1	HIST1H3G	CUL2	EZH2	HIST1H4B	TUBG1
UBC	CCDC8	HIST1H3J	PARP1	ACTB	HIST1H4E	PABPC1
GRB2	VCP	HIST1H3H	PAN2	XRCC6	HIST1H4L	RPL6
COPS5	BRCA1	HIST1H3B	U2AF2	CUL4B	HIST2H4A	DHX9
CUL7	VCAM1	HIST1H3D	PRKDC	FLNA	HIST1H4D	ILF2
CUL1	EED	HIST1H3A	SUZ12	RPL10	HIST1H4F	RPL5
HSP90AA1	CDC5L	HIST1H3F	CUL5	RACK1	HIST1H4K	RPS8
RNF2	SNW1	EWSR1	YWHAE	H2AFX	HIST1H4J	RAD21

any two of the “Drug-likeness” cells. After obtaining the screened active ingredients from the antlers, they were imported into SwissTargetPrediction to predict the drug targets, and those with  $P$  values  $>0$  were selected, validated, and supplemented in the UniProt database.

**2.2. IVDD Target Collection.** Disease-related genes were searched for in GeneCards, TTD, DrugBank, DisGeNET, OMIM, and other disease target databases using “IVDD” as the search term and screened for human race. Subsequently, the collected disease targets were



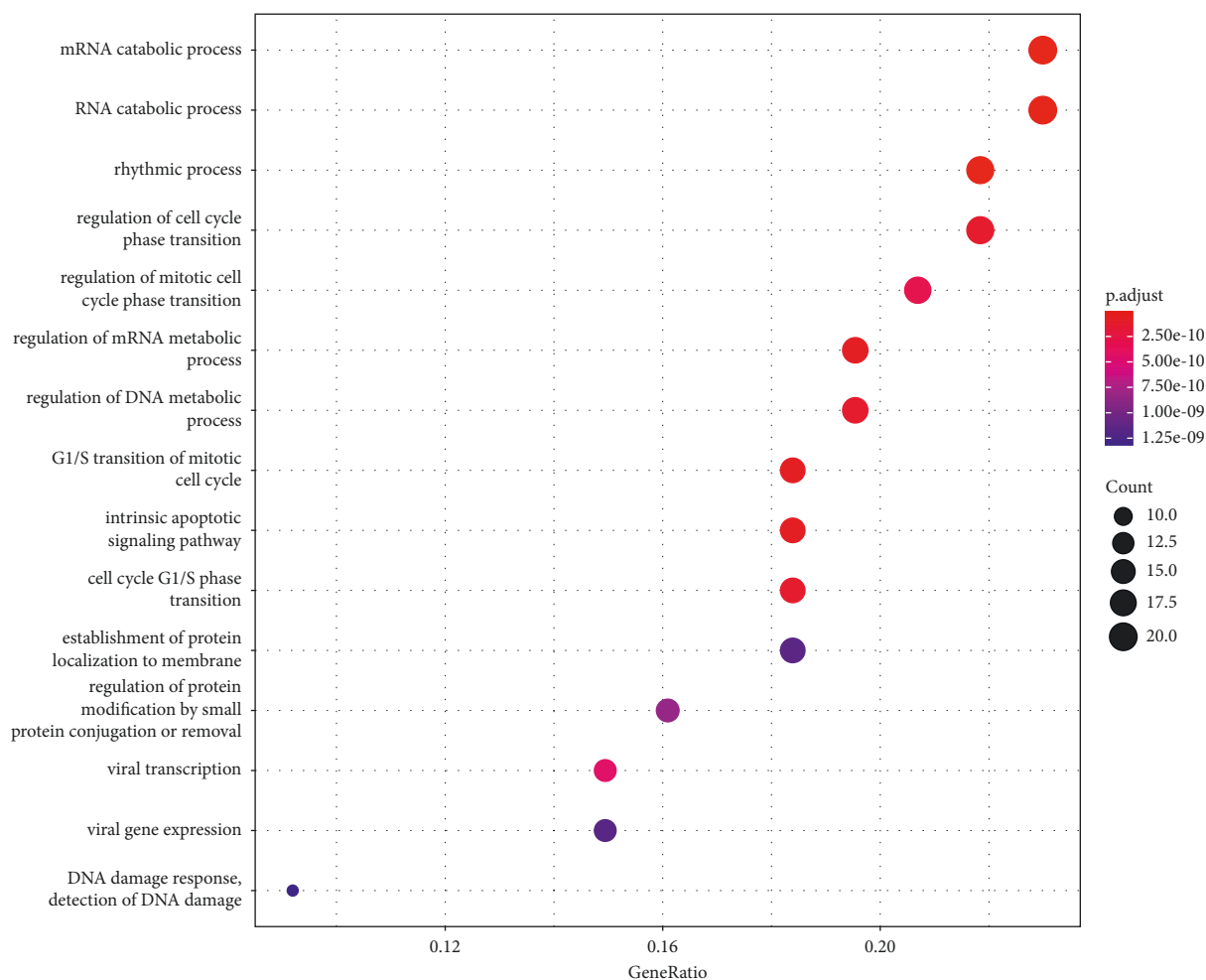


FIGURE 4: Significant bubble diagram of a biological process.

aggregated and deduplicated, and they were standardized and supplemented with gene names in the UniProt database.

**2.3. Core Target Screening.** Using the BisoGenet plug-in in Cytoscape 3.7.2, protein interaction networks were predicted separately for drug component targets and disease targets, and the two networks were merged to collect their intersecting targets. Next, the CytoNCA plug-in was used to analyze the topology of the intersection network and extract the HUB targets based on the values of “Betweenness,” “Degree,” and “Closeness” in the results as filtering criteria.

**2.4. GO and KEGG Enrichment Analyses.** GO enrichment analysis is able to discover the link between genes and gene product features across all species. KEGG enrichment analysis is useful in clarifying in vivo comprehensive inferences of reactions. The R software packages “pathview” and “clusterProfiler” were used for KEGG and GO enrichment analyses of intersection targets. Finally, the “ggplot” software package was used to visualize the results.

**2.5. Molecular Docking.** Five core target proteins in the PPI network and significant components in the “component-target” network diagram were selected for molecular docking verification. The 3D structures of the target protein were downloaded from the PDB website (<https://www.rcsb.org/>), and the 3D structures of the components were obtained from the ZINC website (<https://zinc.docking.org/>). First, the intrinsic small ligand of the protein was docked to the protein structure, and the binding energy was calculated. Second, the target proteins and components were docked in AutoDock Vina (v1.1.2), and the binding energy was also calculated. The binding energy of the intrinsic small ligand to the protein was the standard for verifying the binding conditions of the new compounds. Ultimately, PyMOL (v2.5) was utilized to present a visual analysis of the molecular structure. Figure 1 shows the brief flowchart with network pharmacology and molecular docking.

### 3. Result

**3.1. Component-Target Network Diagram Construction.** A total of 5 active ingredients were obtained from the screening of deer antlers (Table 1). A total of 104 targets were

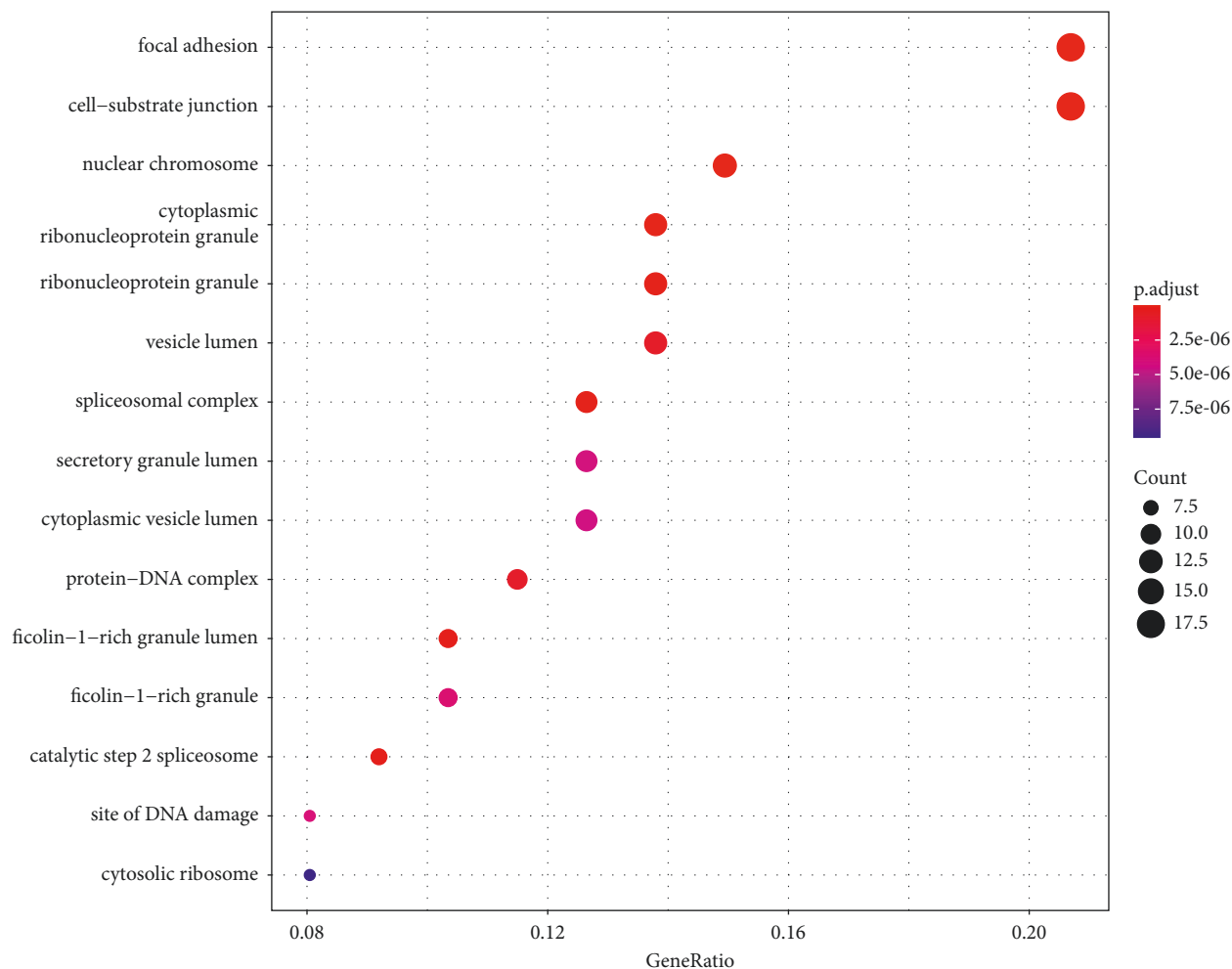


FIGURE 5: Significant bubble diagram of cell components.

obtained after aggregation and deweighting. The data were imported into Cytoscape 3.7.2 to construct a “component-target” network diagram, which showed a total of 104 nodes and 238 component-target interactions (Figure 2). From the figure, we can find that alpha-estradiol and 17-beta-estradiol interact with the most target genes and are potential key compounds. Based on the results of the topological analysis, we attained the degree values for the five active ingredients (Table 2).

**3.2. Results of the Core Target Screening.** A total of 1023 disease targets were obtained after the search summary, and after intersection mapping with drug action targets, the intersection network was topologically analyzed using the CytoNCA plug-in. Subsequently, the “Degree” value in the results was used as a filtering criterion to screen targets with a “Degree” value greater than or equal to 2 times the median (degree  $\geq 68$ ), and a total of 2579 targets were obtained, that is, because the degree value can reflect the importance degree of a compound in deer antlers. Afterward, the new results were analyzed according to the three indicators “Betweenness,” “Degree,” and “Closeness,” and targets greater than or equal to the median were screened, resulting in 112 core targets (Figure 3, Table 3).

**3.3. GO and KEGG Enrichment Analyses Results.** A total of 1061 GO entries were obtained after enrichment, containing biological process (BP), cellular component (CC), and molecular function (MF), with a  $P$  value screening for each component. The main results are the rhythmic process, mRNA catabolic process, and G1/S transition of the mitotic cell cycle (Figures 4–6). Meanwhile, the core targets were subjected to the KEGG pathway enrichment analysis to screen for pathways with  $P < 0.05$ , and a total of six were obtained, such as the PI3K-Akt signaling pathway, the thyroid hormone signaling pathway, the Notch signaling pathway, the FoxO signaling pathway, the estrogen signaling pathway, and the HIF-1 signaling pathway (Figure 7), among which the specific mechanism of deer antler treating intervertebral disc degeneration in the PI3K-AKT signaling pathway is shown in Figure 8.

**3.4. Molecular Docking Analysis.** As can be found from the PPI interaction analysis diagram, NTRK1, ESR1, CDK2, CUL3, and TP53 were the proteins with the highest degree values, which were also selected as our core target proteins. We listed the basic information of the target protein and the binding energy of the intrinsic ligand and the protein given in Table 4 (CUL3 had no intrinsic small ligand, so its binding

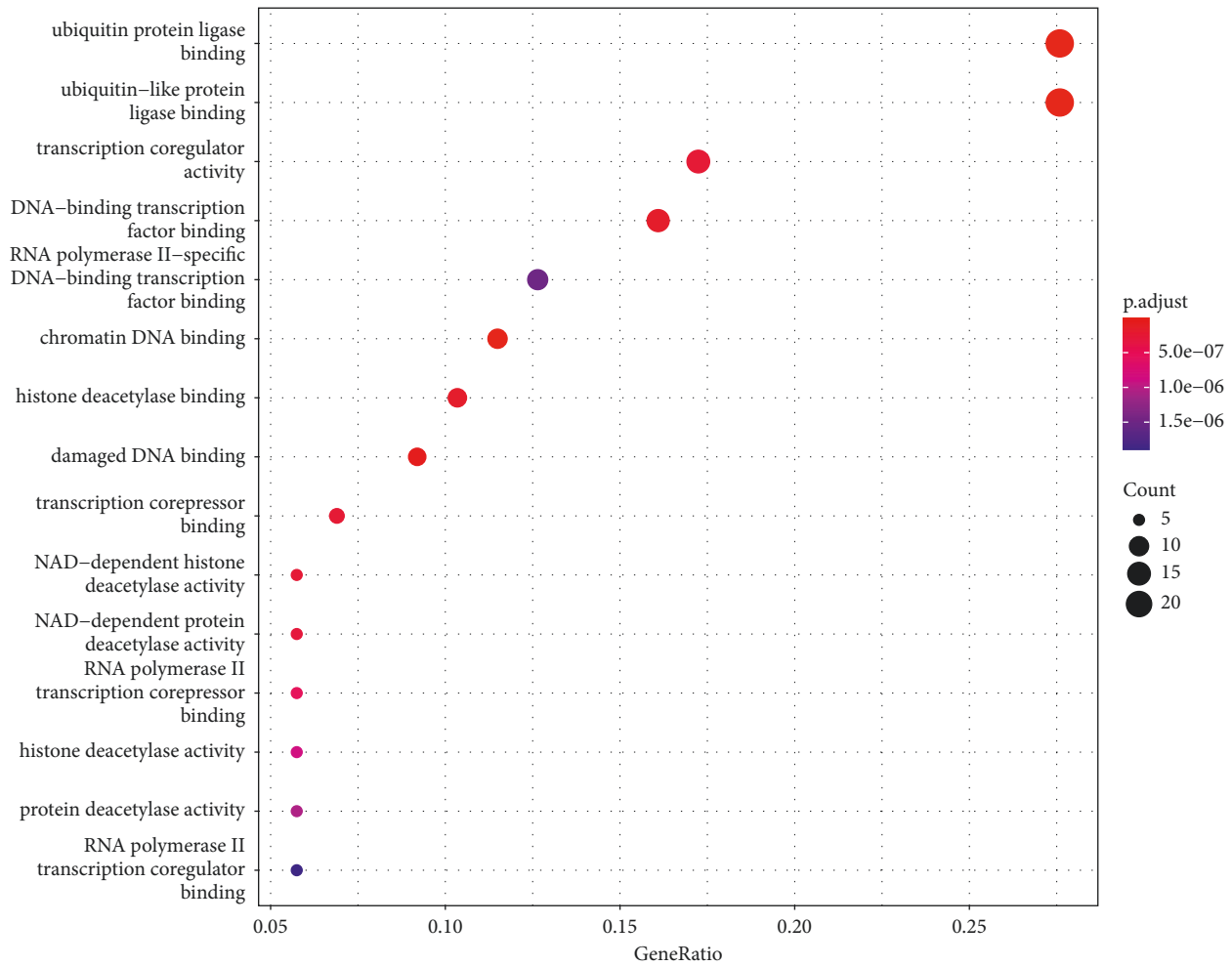


FIGURE 6: Significant bubble diagram of a molecular function.

energy was empty). The binding energies of each component to each target protein were listed in Table 5. The target proteins with the best binding energy were given in Table 6, and the number of hydrogen bonds formed was also recorded. The molecular docking results were presented in Figure 9. The binding energy of estrone with CDK2 was  $-5.9$  kcal/mol. As can be seen from Figure 9, the residues THR-14, THR-158, and estrone formed two hydrogen bonds. Estrone and ILE-35, PRO-45, and PHE-152 formed alkyl interactions (Figure 9(a)). The binding energy of 17-beta-estradiol with NTRK1 was  $-5.5$  kcal/mol. There was no hydrogen bond formed between 17-beta-estradiol with NTRK1. The residue ARG-574 and 17-beta-estradiol formed one Pi-alkyl interaction. 17-Beta-estradiol and HIS-503, LEU-532, and GLN-568 formed alkyl interactions (Figure 9(b)). The binding energy between alpha-estradiol and TP53 was  $-6.85$  kcal/mol. LYS-164 of TP53 and alpha-estradiol formed a hydrogen bond. Alpha-estradiol and GLN-100, SER-166, and Mer-169 formed alkyl interactions. Alpha-estradiol and the residue GLN-167 formed one Pi-alkyl interaction (Figure 9(c)). The binding energy of estrone with CUL3 was  $-4.42$  kcal/mol. CUL3 and the residues SER270 and PRO259 formed two hydrogen bonds. The residues ARG266, VAL263, and estrone formed two Pi-

cation bonds. Estrone and the residues PRO307 and GLU258 formed alkyl interactions (Figure 9(d)). The binding energy between estrone and ESR1 was  $-6.46$  kcal/mol. Estrone and the residue TYP213 formed two Pi-Pi interactions. Estrone and HIS206, GLU210 formed alkyl interactions (Figure 9(e)).

#### 4. Discussion

First recorded in the Shen Nong Ben Cao Jing, deer antlers are true bone growth found in pairs on the heads of male deer and are commonly utilized as Chinese medicine to tonify “kidney-yang,” which is believed to have the effect of strengthening the muscles and bones. Modern pharmacology has found through various experimental studies that deer antlers have significant anti-inflammatory and antioxidant effects, which protect osteoblasts, and this may be the mechanism by which deer antlers treat degenerative disc degeneration. However, few studies explored the molecular mechanism of deer antlers in the treatment of IVDD. Through the network pharmacology and molecular docking analysis of deer antlers for the treatment of IVDD, we have made a preliminary exploration of its molecular mechanism.

The mechanisms by which IVDD occurs are complex, and current studies mainly focus on oxidative stress [17],

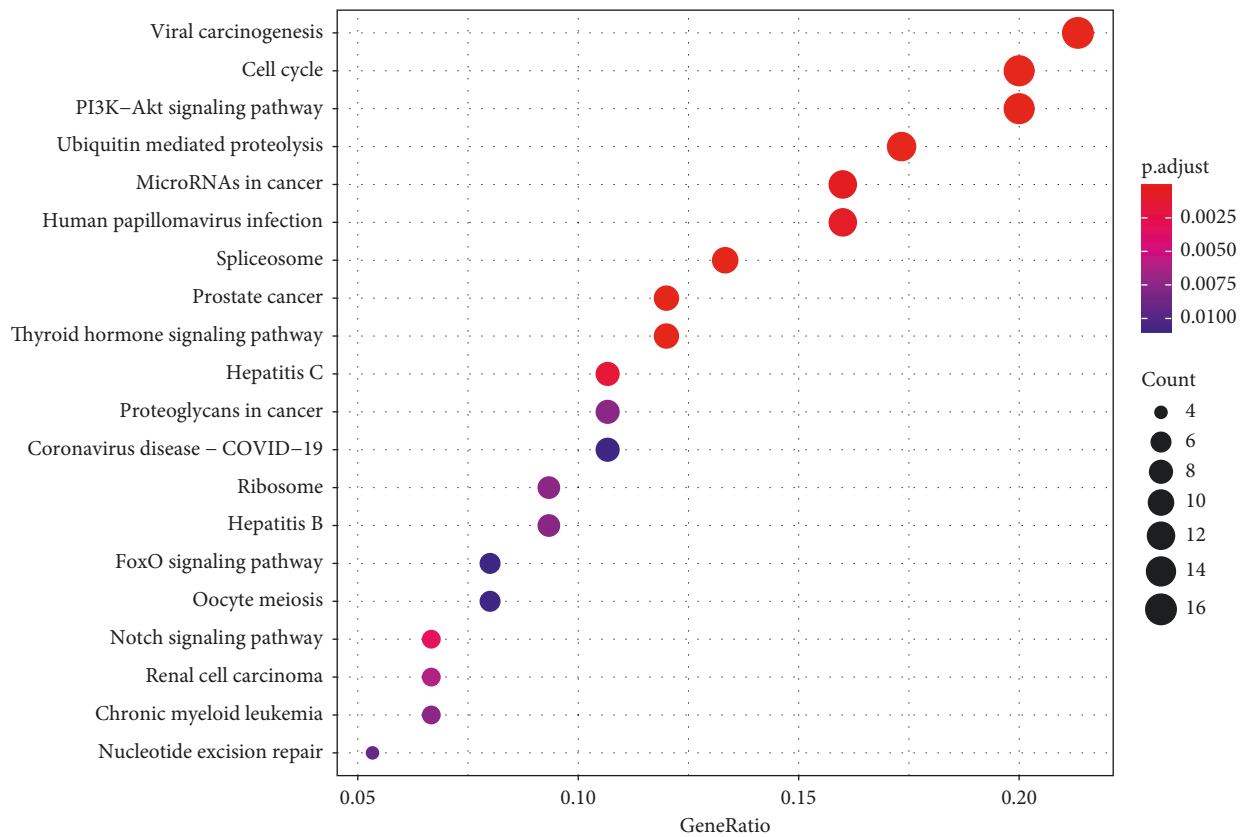


FIGURE 7: KEGG enrichment results.

inflammatory irritation [18, 19], nutritional deficiencies [20], and DNA damage [21]. In the screening results of the small molecule active compounds of deer antlers, we found that the active compounds such as estradiol and estrone are all steroid hormones. It has been found that both  $\alpha$ -estradiol and 17- $\beta$ -estradiol can increase the activity of anti-inflammatory markers such as IL-6 receptors through the action of estrogen receptors, thereby reducing the secretion of inflammatory factors TNF- $\alpha$  and IL-6 and inhibiting inflammatory stimulation [22, 23]. It has also been shown that estradiol protects tissues by inhibiting oxidative stress in organ tissues [24, 25]. The specific mechanism of action is through binding to the estrogen receptor. It is particularly noteworthy that some researchers have found that intervertebral discs and their surrounding tissues have a large number of estrogen receptors and that the use of 17-beta-estradiol is effective in slowing down the process of IVDD, which may be a mechanism for the treatment of IVDD with deer antlers [26–28].

All six pathways are crucial to the occurrence of IVDD. The PI3K-Akt signaling pathway plays a critical role in IVDD [29]. After PI3K-Akt activation, it can help promote the apoptosis of nucleus pulposus cells [30]. It was reported by Krupkova that, through promoting Akt phosphorylation, epigallocatechin 3-gallate prevented nucleus pulposus cells from oxidative stress [31]. The thyroid signal pathway is also an important factor. It has been reported that thyroid

hormone can regulate microRNAs to induce an anti-oxidative stress effect [32]. Several studies reported that the Notch signaling pathway can stimulate chondrogenesis and cartilage development, which has an effect on curing IVDD [33]. It is also reported that the activated Notch signaling pathway reduced the growth arrest and apoptosis of nucleus pulposus cells and promoted the regeneration and proliferation of nucleus pulposus cells [34]. The expression of FoxO protein can induce several kinds of antioxidant enzymes and play the role of antioxidation [35, 36]. Alvarez-Garcia O et al. found that IVDD is associated with an obvious decrease in the number of effectors that receive FoxO signals, which affects the expression of FoxO protein [37]. So, we can deduce that Foxo is an important regulatory protein for antiaging and antioxidation. Maintaining the normal expression of FoxO is essential in delaying IVDD. Estrogen secretion can effectively reduce the incidence of IVDD. Some studies have shown that with a reduction in estrogen secretion, postmenopausal women's incidence of IVDD also increased [38]. Other studies have found that the presence of estrogen helps maintain the extracellular matrix [39]. HIF-1 keeps nucleus pulposus cells alive and synthesizes and maintains the extracellular matrix [40]. At the same time, Hif-1 can regulate the stabilization of oxygen by regulating a variety of enzymes [41].

Molecular docking was utilized to determine if the four components had an affinity for the five target



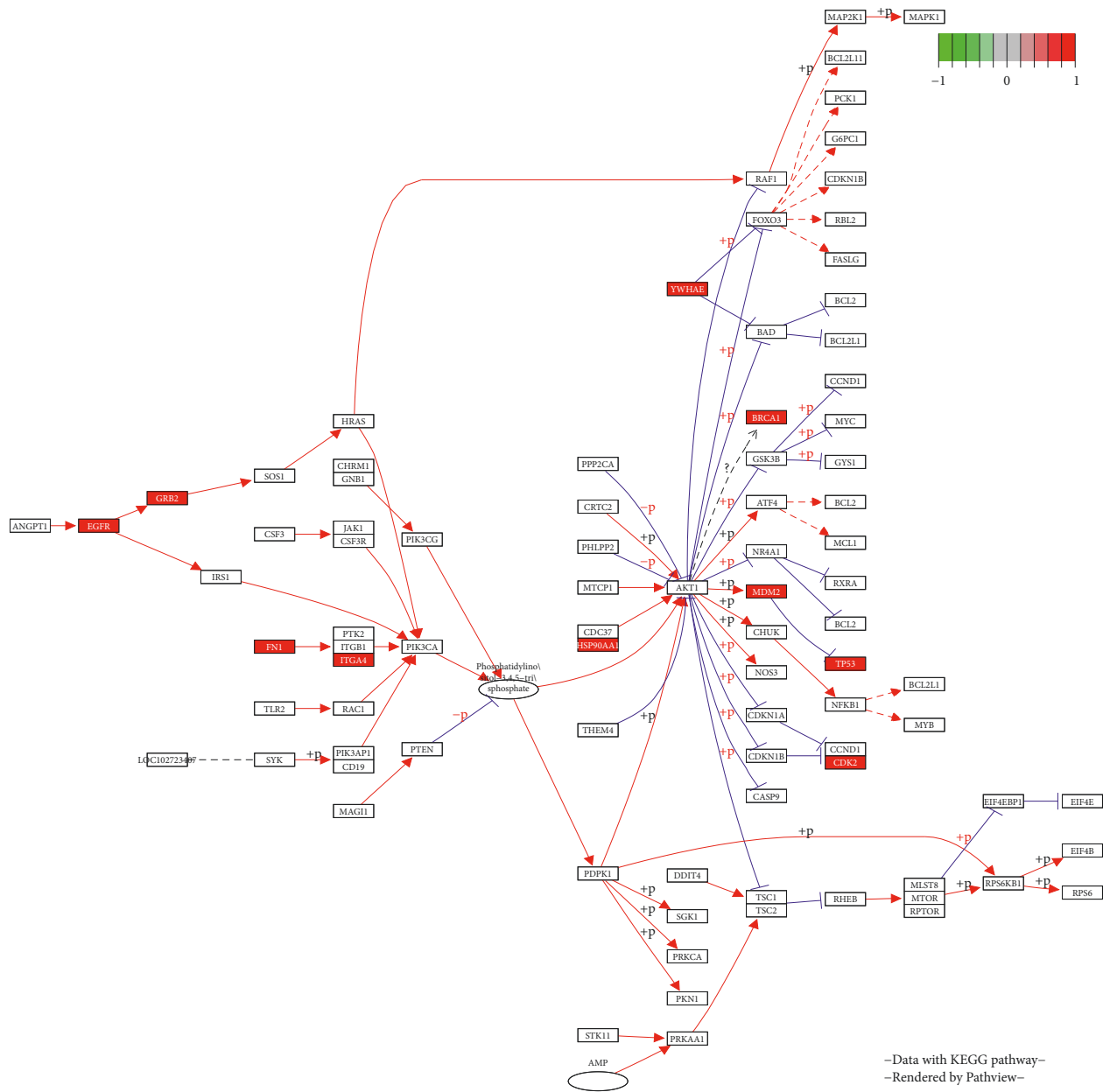


FIGURE 8: PI3K-Akt signaling pathway diagram.

TABLE 4: Basic information of the target protein and the binding energy of the intrinsic ligand.

Target protein	PDB ID	Ligand ID	Binding energy (kcal/mol)
CDK2	2R3R	6SC	-5.83
NTRK1	5JFW	6K2	-12.50
TP53	6GGC	EXN	-9.21
CUL3	6I2M	—	—
ESR1	7B9R	T4Q	-5.23

proteins. With the results listed, it indicates that these components could bind tightly to the target proteins. Given that the binding affinity of alpha-estradiol on TP53 was slightly lower than the original ligand, we can

TABLE 5: The binding energy of each component to each target protein.

Target protein	Estrone	Estragole	Alpha-estradiol	17-Beta-estradiol
CDK2	-5.90	-3.30	-5.12	-5.75
NTRK1	-4.94	-3.23	-4.65	-5.50
TP53	-6.63	-3.45	-6.85	-6.33
CUL3	-4.42	-1.85	-3.02	-4.16
ESR1	-6.46	-2.94	-5.32	-5.66

conclude that estrone on ESR1 had the best docking result. We can speculate that estrone-ESR1 may have a potential antidenaturation effect.

TABLE 6: Target proteins with the best binding energy.

Target protein	Component	Binding energy (kcal/mol)	Number of hydrogen bonds
CDK2	Estrone	-5.90	2
NTRK1	17-Beta-estradiol	-5.50	0
TP53	Alpha-estradiol	-6.85	1
CUL3	Estrone	-4.42	2
ESR1	Estrone	-6.46	0

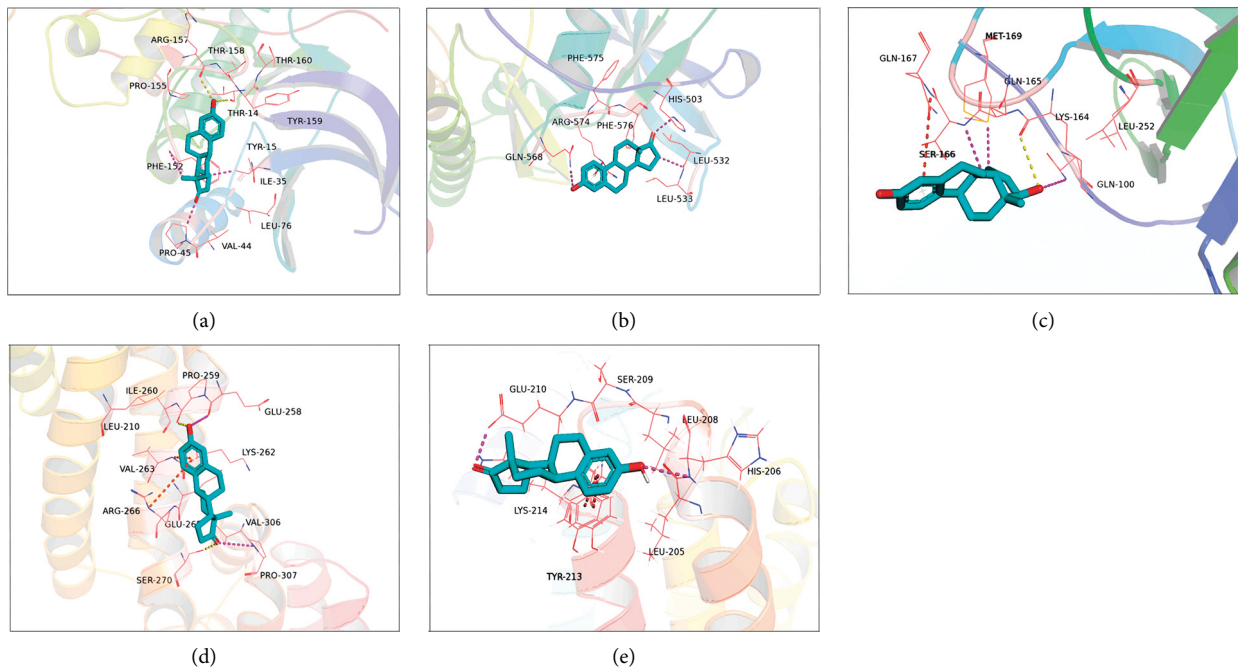


FIGURE 9: Molecular docking of components with target proteins. (a) Molecular docking of estrone with CDK2. (b) Molecular docking of 17-beta-estradiol with NTRK1. (c) Molecular docking of alpha-estradiol with TP53. (d) Molecular docking of estrone with CUL3. (e) Molecular docking of estrone with ESR1.

## 5. Conclusion

In summary, we initially explored the molecular mechanism of deer antlers for the treatment of IVDD through network pharmacology and molecular docking in this study, predicting that the key therapeutic mechanism is anti-inflammatory and antioxidant. We also found that estrone showed the best docking results against ESR1 and predicted that it could be used as a potential drug treatment site. Although the effective compounds from this study screen scored highly, it cannot be determined that the remaining compounds are not clinically effective, especially large-molecule compounds such as peptides. Furthermore, experimental validation of the results of this study is still required due to algorithmic limitations and database sources limited to published research reports.

## Abbreviations

IVDD: Intervertebral disc degeneration  
TCMSP: Traditional Chinese medicine systems pharmacology

PPI: Protein-protein interaction  
GO: Gene ontology  
KEGG: Kyoto Encyclopedia of Genes and Genomes  
TCM: Traditional Chinese medicine.

## Data Availability

The data used to support the conclusions of this study are available from the corresponding author upon request.

## Conflicts of Interest

The authors declare that there are no conflicts of interest.

## Authors' Contributions

Rui Weng and Wenchao Li designed and conceptualized the study and revised the manuscript. Rui Weng, Hongheng Lin, and Wenchao Li wrote the manuscript. Xiaoxiao Lin, Zhenyu Zhang, Qiqi Chen, and Yiqi Yao were responsible for extracting data. Rui Weng, Zhuoyao Li, and Daman Chen carried out the molecular docking analysis.

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