Hindawi
Journal of Food Biochemistry
Volume 2023, Article ID 1468967, 11 pages
https://doi.org/10.1155/2023/1468967

Research Article

Network Pharmacology Analysis and Experimental Pharmacology Study Explore the Mechanism of *Asparagus* against Glioblastoma

Xin Luo,[1] **Jinshan Xing**,[2] **Yingjiang Gu**,[2] **Gan Qiao**,[1] **Minghua Liu**,[1] **and Jingyan Yi**[3]

[1]*Department of Pharmacology, School of Pharmacy, Southwest Medical University, Luzhou 646000, Sichuan, China*
[2]*Department of Neurosurgery, The Affiliated Traditional Chinese Medicine Hospital of Southwest Medical University, Luzhou 646000, Sichuan, China*
[3]*Department of Medical Cell Biology and Genetics, School of Basic Medical Sciences, Nucleic Acid Medicine of Luzhou Key Laboratory, Key Laboratory of Medical Electrophysiology, Ministry of Education & Medical Electrophysiological Key Laboratory of Sichuan Province, (Collaborative Innovation Center for Prevention of Cardiovascular Diseases), Institute of Cardiovascular Research, Southwest Medical University, Luzhou 646000, Sichuan, China*

Correspondence should be addressed to Jingyan Yi; jingyany@swmu.edu.cn

Received 19 October 2022; Revised 12 November 2022; Accepted 13 December 2022; Published 8 February 2023

Academic Editor: Swapan Ray

Glioblastoma (GBM) is the most malignant primary brain tumor, and there is an urgent need to explore effective therapeutic strategies to improve the prognosis of GBM patients. *Asparagus* (ASP) is a widely grown plant with a rich pharmacological profile that has been used to treat various cancers. However, its role in GBM remains unclear. In the study, we confirmed the inhibitory effect of ASP on GBM and explored the target and mechanism of ASP through network pharmacology, molecular docking, and molecular biology experiments. We found that P53 is a potent target of ASP for GBM treatment, and its pharmacological mechanism is the activation of p53-dependent apoptosis. Our research provides an objective basis for the development and clinical application of ASP in GBM patients.

1. Introduction

Glioma is the most common primary tumor of the central nervous system, and glioblastoma (GBM) is one of the most malignant gliomas [1]. GBM is highly heterogeneous and aggressive, with a high incidence and a poor prognosis [2]. The current treatment for GBM is mainly surgery combined with radiotherapy and chemotherapy [2]. Unfortunately, these treatments did not significantly improve the median survival of patients, mainly due to treatment resistance and tumor recurrence after surgical resection [3]. Therefore, there is an urgent need to explore effective treatment strategies and drugs to improve the prognosis of GBM patients.

Traditional Chinese medicine (TCM) has a high status not only in China but also in the world [4]. TCM has received widespread attention for its effectiveness and low side effects in treating cancer patients [5]. *Asparagus* (ASP) is a tuberous vegetable of the lily family, whose roots and shoots are rich in many biologically active phytochemicals, including oligosaccharides, steroidal saponins, amino acid derivatives, and essential minerals, and has been used to treat various cancers [6–8]; Zhang et al. [9]. However, the role of ASP in GBM has not been reported, and its pharmacological mechanism deserves further exploration.

As a brand-new discipline based on systems biology, bioinformatics, and high-throughput histology, network pharmacology has achieved remarkable results in exploring the therapeutic mechanisms, screening active ingredients, and therapeutic targets of TCM [10]; Zhang et al. [9]. In this study, we explored the potential mechanisms of ASP for GBM based on network pharmacology, molecular docking, and experimental validation. We found that ASP achieves

the therapeutic effect of GBM by activating P53 and inducing p53-dependent apoptosis. The workflow is shown in Figure 1.

2. Materials and Methods

2.1. Network Pharmacology. The systematic pharmacology database of traditional Chinese medicine (TCMSP, https://tcmspw.com/tcmsp.php) was used for screening active ASP compounds [11]. For qualified herbal compounds, oral bioavailability (OB) and drug-like (DL) were set to ≥30% and ≥0.18, respectively, using the TCMSP platform's pharmacokinetic information retrieval filter. Chemical structures of the corresponding compounds were downloaded using the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). GeneCards (https://www.genecards.org/) and OMIM (https://www.omim.org/ updated in 2022) databases were used to predict and screen GBM targets. ASP and GBM-related targets were screened for common targets using the VennDiagram package in R.

In this study, a drug compound-disease-target network and a drug compound-disease-pathway-target network were constructed using the Cytoscape V 3.7.0 software (https://www.cytoscape.org/) [12]. Protein-protein interaction (PPI) networks for common ASP and GBM targets were constructed using the STRING database (https://cn.string-db.org/) [13]. Top 10 PPI network proteins visualized with Cytoscape V 3.7.0 software based on medium-confidence target protein interaction data (score >0.4). We analyzed Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways using clusterProfiler in R. A tool was used to produce the intersection selection of differential genes in crucial pathways (https://bioinformatics.psb.ugent.be/webtools/Venn/).

2.2. Molecular Docking. Molecular docking simulations were utilized to validate the binding of targets and corresponding compounds. We obtained data on macromolecular protein target receptors from the RCSB PDB database (https://www.rcsb.org/) and data on small molecule compounds from the PubChem database and TCMSP [14]. PyMOL 3.7 software was used to expel water and ligand from macromolecular proteins downloaded from PDB [15]. The software AutoDockTool 1.5.7 was used to perform docking simulations of macromolecular protein targets and their corresponding compounds [16]. The search parameters were determined using a genetic algorithm, followed by network visualization using PyMOL 3.7 software.

2.3. Cell Cultures. GBM cells were purchased from iCell Bioscience Inc. (China). Cells were maintained in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum and penicillin/streptomycin (100 units/ml) at 37°C and 5% CO_2.

2.4. Cell Viability Assay. We diluted the ASP extract (10 : 1) in purified water to 2 g/ml, filtered it through a 0.22 m filter, and stored it at 4°C. Cell viability was assayed using the cell counting kit-8 (CCK8) assay according to the manufacturer's protocol (Dojindo Molecular Technologies, Japan).

2.5. Clonogenic Assay. GBM cells were seeded on plates and cultured for 24 h before the initiation of therapy. Fresh media were replaced every 3 days. At the endpoint, cells were washed with phosphate-buffered solution and subsequently stained with 5% crystal violet for 1 h. A Molecular Imager (USA) was used to capture images of stained plates. A multifunctional microplate reader was used to measure the optical absorbance of bound crystal violet (dissolved in 50% acetic acid) at 570 nm.

2.6. Western Blot Analysis. Fresh cells were lysed with RIPA lysis buffer. Protein was separated by SDS-PAGE and transferred to PVDF membranes. Antibodies against P53 (AF0255, 1 : 1000), PARP/cleaved-PARP (WL01932, 1 : 500), caspase-3/cleaved caspase-3 (WL02117, 1 : 500), cleaved caspase-8 (#8592, 1 : 1000), BCL2 (#3498, 1 : 1000), BAX (#41162, 1 : 1000), AKT1 (AF0045, 1000), p-AKT (#4060, 1 : 1000), p-S6RP (#4858, 1 : 1000), p-4EBP1 (#2855, 1 : 1000), cleaved caspase-9 (WL01838, 1 : 500), and β-actin (66009-1-Ig, 1 : 10000) were used as the primary antibodies. HRP-conjugated antibodies against mice or rabbits (1 : 10000, ProteinTech Group, Inc.) were used as the secondary antibodies. Immunoblot imaging was performed using the BIO-RAD ChemiDoc™ XRS + Molecular Imager. The Western blot was normalized to β-actin.

2.7. Apoptosis Analysis. Hoechst 33342/PI dual staining kit (Solarbio, China) was used to detect apoptosis in cells. Images were taken with fluorescence microscopy (Olympus, Japan). Apoptotic cells were stained with red fluorescence, and all cells had blue fluorescence in their nuclei.

2.8. Flow Cytometry Analysis. Apoptosis in GBM cells was analyzed with the Annexin V-FITC Apoptosis Detection Kit (Beyotime, China) according to the manufacturer's instructions. Briefly, cultured cells were trypsinized with 0.25% trypsin without EDTA and then stained with annexin V-FITC and propidium iodide (PI) solutions. Stained cells were subjected to flow cytometry analysis on a BD FACS Aria II (BD Biosciences, USA).

2.9. Statistical Analyses. All data are expressed as mean ± SEM. Statistical analysis was performed using GraphPad Prism 8 software. A Student *t*-test was used to compare differences between two independent groups. *P* values less than 0.05 were considered statistically significant and are denoted as follows: $^{*}0.05$, $^{**}0.01$, and $^{***}0.001$.

3. Results

3.1. Screening of the Candidate Genes of the Active Compounds in ASP. The TCMSP database was used to obtain 9 components in ASP based on OB and DL conditions, respectively: beta-sitosterol, sitosterol, methylprotodioscin_qt,

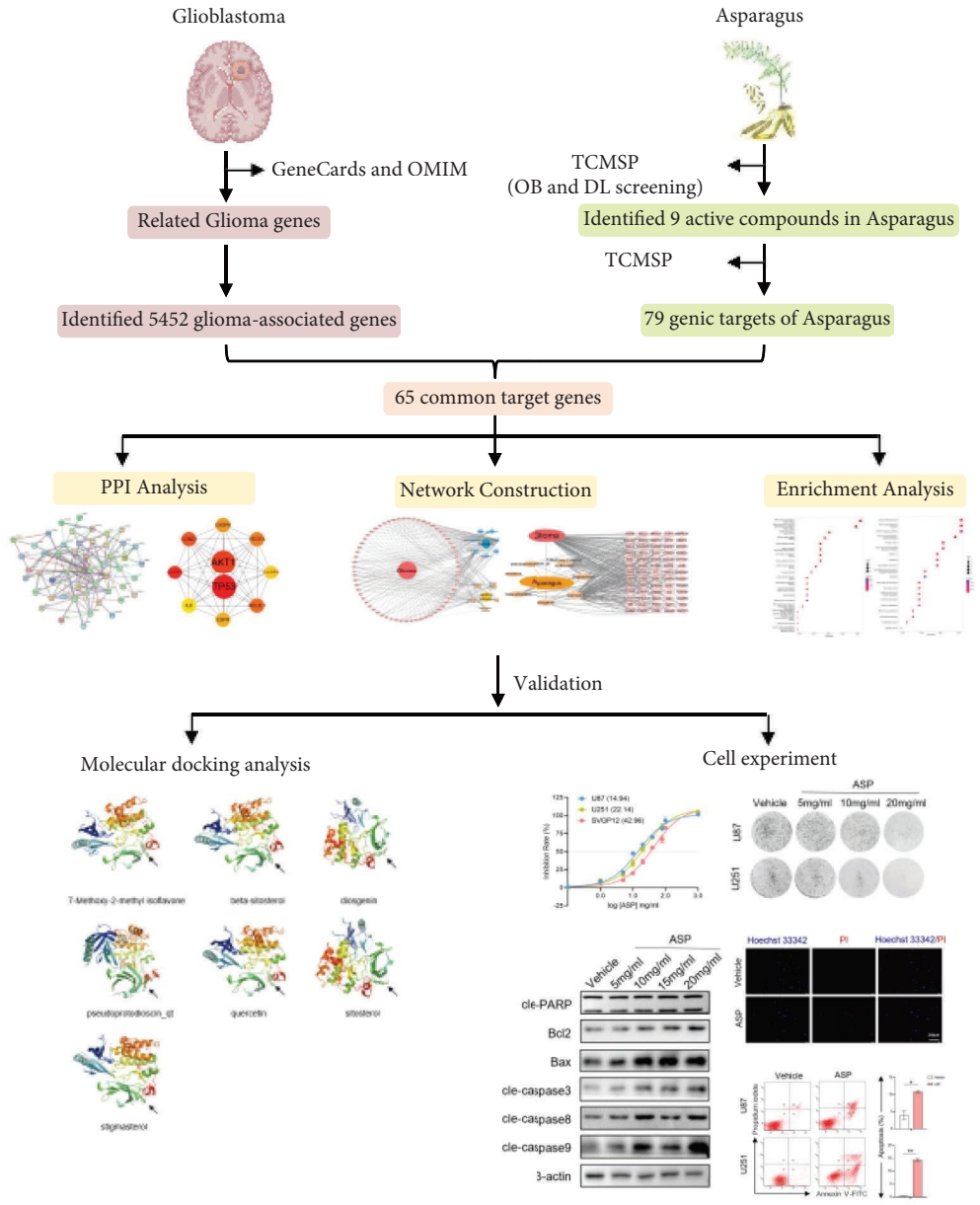


Figure 1: Schematic representation of the proposed mechanism in ASP against GBM. Network pharmacology was applied to analyze the crucial components and key targets of ASP for the treatment of GBM. Cellular assays confirmed that ASP reduced the proliferative capacity of GBM cells and induced cell apoptosis through a p53-dependent pathway.

pseudoprotodioscin_qt, 7-methoxy-2-methyl isoflavone, Asparaside A_qt, stigmasterol, diosgenin, and quercetin (Table 1). The 79 target genes in 7 components were screened (methylprotodioscin_qt and Asparaside A_qt did not have a target gene).

3.2. Screening of Candidate Targets for GBM. Using GBM as an index keyword, we mined 5435 and 31 genes associated with GBM from GeneCards and OMIM databases, respectively. With duplicates removed and search results combined, a total of 5452 GBM-associated genes were identified. By intersecting ASP key component target genes with disease-associated genes, we found 65 ASP-GBM common targets (Figure 2(a)).

3.3. Network Pharmacology Analysis of ASP in GBM. To visualize compound-disease-target networks, we imported 7 active ingredients and 65 drug-disease key target genes into Cytoscape 3.7.0 (Figure 2(b)). It can be seen that the active ingredient with the most target genes in ASP is quercetin. To investigate how these genes are related to each other, we constructed a PPI network using the STRING online

Table 1: ASP active components list.

Molecule name	Mol ID	Oral bioavailability (OB) (%)	Drug-like (DL)
Beta-sitosterol	MOL000358	36.91	0.75
Sitosterol	MOL000359	36.91	0.75
Methylprotodioscin_qt	MOL003889	35.12	0.86
Pseudoprotodioscin_qt	MOL003891	37.93	0.87
7-methoxy-2-methyl isoflavone	MOL003896	42.56	0.20
Asparaside A_qt	MOL003901	30.60	0.86
Stigmasterol	MOL000449	43.83	0.76
Diosgenin	MOL000546	80.88	0.81
Quercetin	MOL000098	46.43	0.28

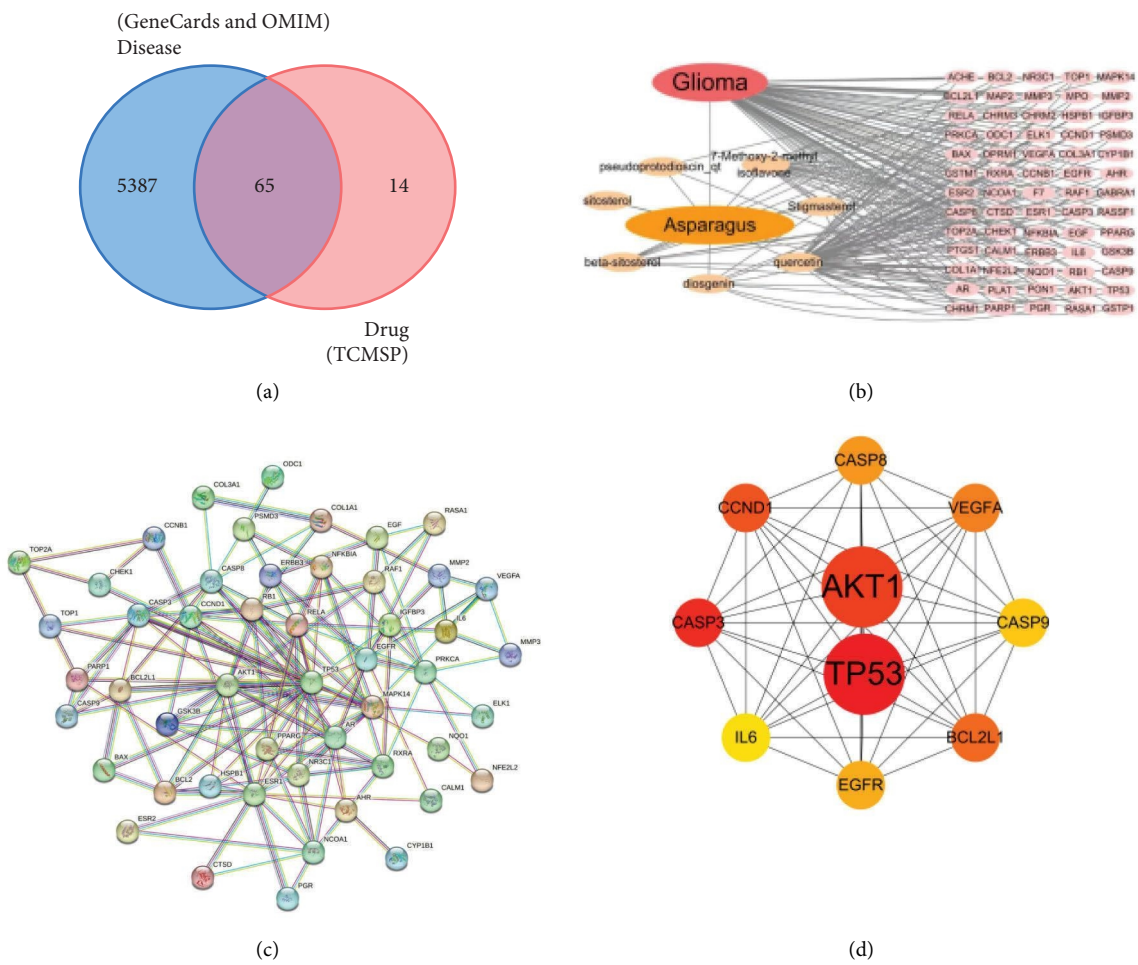


Figure 2: Network pharmacological analysis of ASP for GBM. (a) Disease-drug Venn diagram. (b) Composition-target-disease network of ASP. The deep red oval represents the disease; orange ovals represent the compound component; pink ovals represent targets. (c) Visualization analysis of the target PPI network. (d) The 10 hub genes were identified from the PPI network (score >0.4). Genes are more significant when the color is darker.

database (Figure 2(c)). The interaction network was then imported into the Cytoscape software. Finally, we screened the top 10 genes using the MCC algorithm of the cytoHubba plugin, which included two important genes: AKT1 and P53 (Figure 2(d)).

3.4. Molecular Docking Simulates the Binding of AKT1 and P53 with ASP Active Ingredients. AKT1 and P53 are both genes that are closely related to tumor research [17]. Based on docking simulations of AKT1 and P53 with the 7 active components, we explored their association with ASP. The

binding ability is shown in Table 2, and the binding energy of P53 is lower compared to AKT1, suggesting a better binding ability. The binding schematic is shown in Figures 3 and 4.

3.5. Pathway and Process Enrichment Analysis. We then performed a functional annotation to explore the effects of ASP using KEGG pathways and GO biological processes. Based on the 109 GO analyses, DNA-binding transcription factor binding ranked highest, suggesting that ASP may play an important role in the nucleus. (Figure 5(a)). Having analyzed 145 KEGG pathways, we speculate that ASP may act through chemical carcinogenesis—receptor activation (Figure 5(b)). The top 5 KEGG pathways filtered according to p value and count numbers were the PI3K-Akt signaling pathway, lipid and atherosclerosis, hepatocellular carcinoma, apoptosis, and p53 signaling pathway (Figure 5(c)). Interestingly, we found that AKT1 and P53 are also central to the above pathways when crossing over a set of genes (Figure 5(d)). A visualized compound-disease-target-pathway network was constructed using KEGG pathways, active ingredients, and 65 targets (Figure 5(e)).

3.6. AKT1 May Not be an Effective Target for ASP in the Treatment of GBM. To verify the therapeutic effect of ASP on GBM, we performed a half-inhibitory concentration assay in normal glial cells and GBM cells. The results showed that the IC50 of ASP in GBM cells was smaller than that of normal cells, suggesting that ASP was more sensitive to GBM (Figure 6(a)). The clonogenic assay showed a concentration-dependent therapeutic effect of ASP on both GBM cells (Figure 6(b)). After that, we examined the changes in AKT1, one of the predicted targets of ASP in GBM cells. Unfortunately, in our study, both high and low concentrations of ASP did not affect AKT1 expression (Figures 6(c) and 6(d)). Consistent with previous studies, the abundance of p-AKT, p-S6RP, and p-4EBP1 proteins (downstream effectors of PI3K/AKT/mTOR signaling) also did not change significantly (Figures 6(c) and 6(d)). The above results suggest that AKT1 may not be an effective target for ASP treatment of GBM.

3.7. Apoptosis is Induced by ASP by Activating P53. We then investigated whether ASP could affect the expression of P53. With increasing drug concentration, P53 was significantly activated (Figure 7(a)). Based on pathway enrichment, we found a significant intersection between the p53 signaling pathway and the apoptotic pathway, where BCL2, BAX, CASP3, etc. are important proteins for apoptosis (Figure 7(b)). This suggested to us that ASP may affect p53-dependent apoptosis. Thus, we examined the expression of apoptosis-related proteins after ASP treatment in the U87 and U251 cell lines. The results showed that cleaved-PARP and cleaved-caspase-3 were significantly enhanced, while BCL2/BAX were significantly decreased, indicating that GBM cells underwent apoptosis (Figures 7(c) and 7(d)). Meanwhile, we further verified that ASP caused apoptosis in GBM cells using Hoechst 3342 and PI double-staining assays. As a result of ASP treatment, the red foci on the cells significantly increased, indicating that apoptotic cells were being generated (Figures 7(e) and 7(f)). This was also confirmed by a flow cytometric Annexin V/PI assay. ASP can significantly increase late apoptosis in GBM cells (Figure 7(g)).

4. Discussion

GBM is the most common and aggressive type of primary brain tumor. Traditional treatment options for GBM, including surgical resection, radiotherapy, and chemotherapy with temozolomide, are considered to have a poor prognosis [1, 2]. The growth characteristics of GBM cells make it difficult to completely remove tumor tissue during surgery [18]. The median survival time of patients after surgery with concurrent radiotherapy and chemotherapy is 12–15 months, but the 5-year survival rate is still less than 5% [2]. Therefore, one of the greatest therapeutic challenges in modern medicine is how to improve the treatment outcome of GBM.

Due to its multitargeted nature, TCM is a very effective approach to treating a wide range of diseases [4, 19]. It has been reported that polysaccharide, saponin, and flavonoid extracts from ASP, a tuberous vegetable of the lily family, are anticancer agents in lung and bladder cancers [6, 20]. As a result of ASP extracts exerting prooxidant activity on breast tumor cells, they have a combined lethal effect with menaquinone [21]. The methanolic extract of ASP, however, could regulate apoptosis by activating the TRAIL death receptor pathway in colon adenocytes [22].

Network pharmacology has been widely used to understand the complex mechanisms of drug therapy [23]. In this study, network pharmacology and experimental validation were applied to explore the material basis of ASP and the potential molecular mechanisms behind its treatment. We first identified 9 active ingredients of ASP and screened 5387 therapeutic targets related to GBM by data mining. As one of the active ingredients of ASP, quercetin closely adheres to the therapeutic targets of GBM, and it may be responsible for GBM's therapeutic effects. Plant-derived quercetin is a flavonoid with anticancer and antiproliferative properties [24]. According to multiple lines of evidence [25, 26], a variety of proteins involved in GBM cell signaling are regulated by quercetin.

In the present study, we screened the potential targets of ASP for GBM treatment, AKT1, and P53, through data mining, and the molecular docking results showed that ASP has a vigorous affinity with both of them. However, we only observed a regulatory effect of ASP on P53 in GBM cell experiments. In addition, we did not observe changes in downstream effector proteins of PI3K/AKT/mTOR, suggesting that AKT1 may not be an effective target for ASP treatment. After further analysis of the pathway enrichment results, we found that there was a large overlap between the p53 signaling pathway and the apoptotic pathway genes. We then found that ASP significantly caused apoptosis in GBM cells and that key proteins of p53-dependent apoptosis all

Table 2: Molecular docking score.

Molecule name	Docking score (kcal/mol)	Protein
Beta-sitosterol	−6.90	AKT1
Sitosterol	−7.01	AKT1
Pseudoprotodioscin_qt	−6.36	AKT1
7-methoxy-2-methyl isoflavone	−5.29	AKT1
Stigmasterol	−7.31	AKT1
Diosgenin	−7.02	AKT1
Quercetin	−5.29	AKT1
Beta-sitosterol	−7.88	P53
Sitosterol	−7.76	P53
Pseudoprotodioscin_qt	−7.13	P53
7-methoxy-2-methyl isoflavone	−6.28	P53
Stigmasterol	−8.05	P53
Diosgenin	−7.66	P53
Quercetin	−6.27	P53

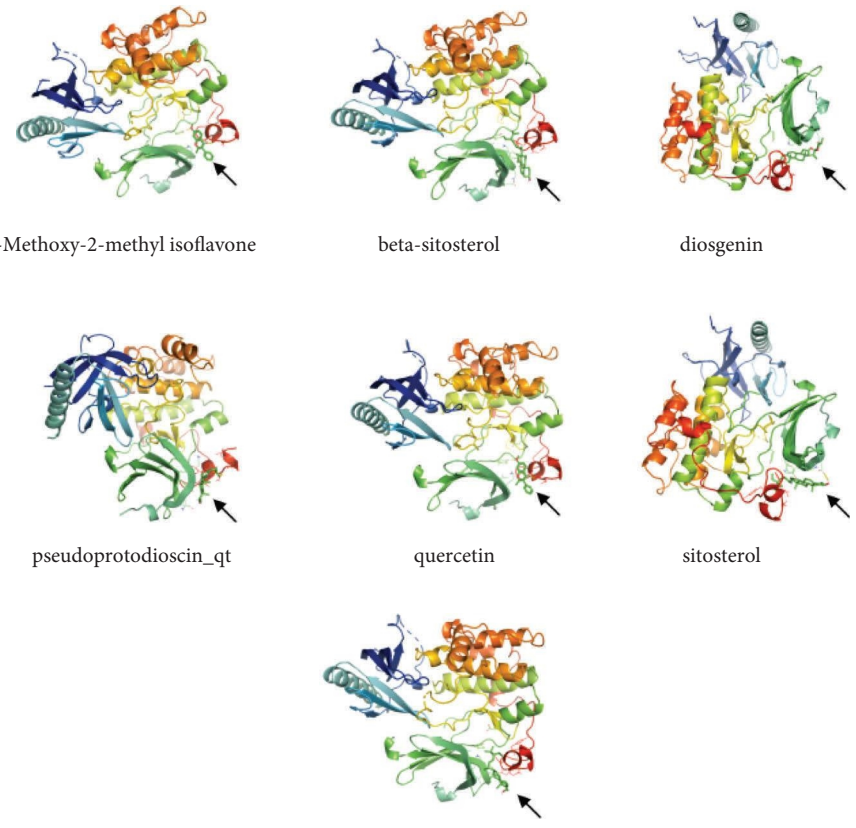


Figure 3: Molecular docking model of AKT1 binding to ASP active components. AKT1 binds to 7-methoxy-2-methyl isoflavone, beta-sitosterol, diosgenin, pseudoprotodioscin_qt, quercetin, sitosterol, and stigmasterol. Black arrows indicate the position of the ligand.

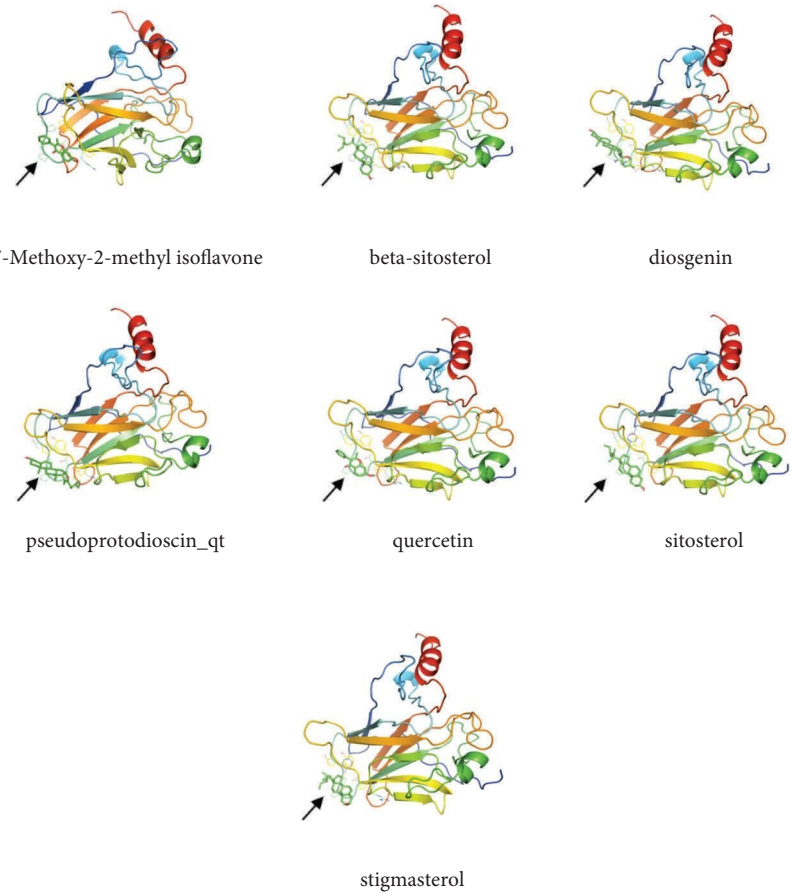


Figure 4: Molecular docking model of the P53 binding to ASP active components. P53 binds to 7-methoxy-2-methyl isoflavone, beta-sitosterol, diosgenin, pseudoprotodioscin_qt, quercetin, sitosterol, and stigmasterol. Black arrows indicate the position of the ligand.

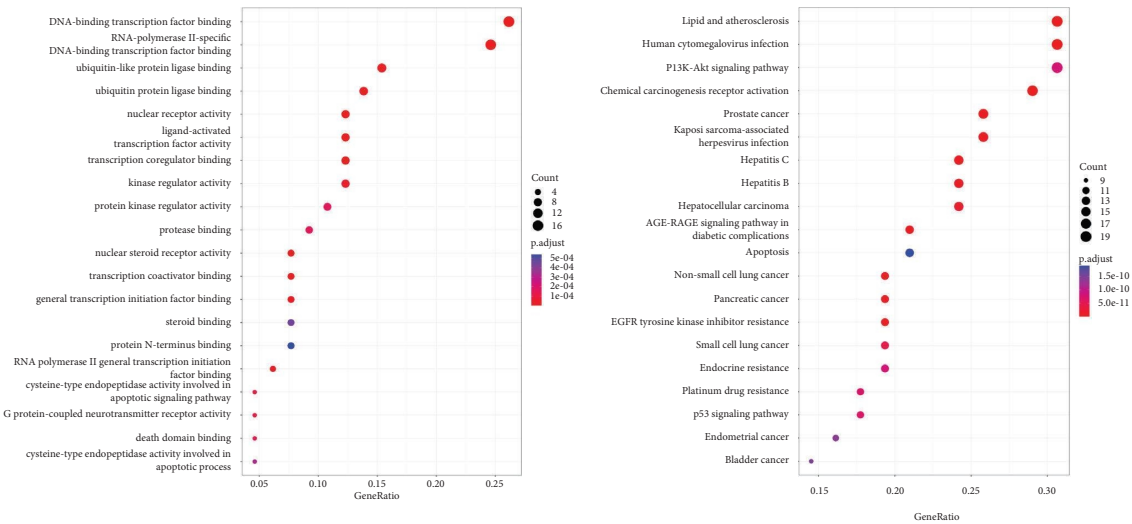
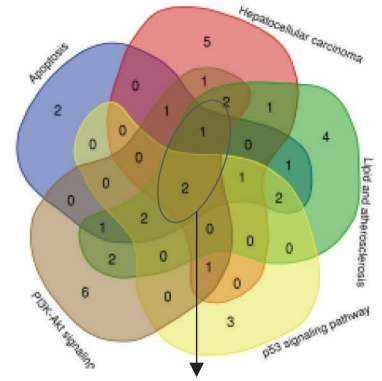
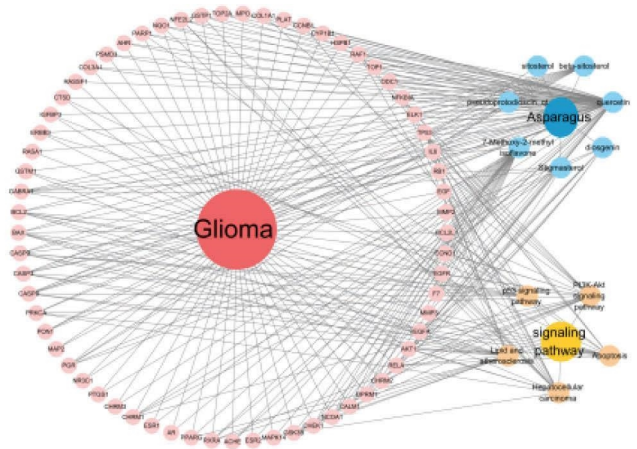


Figure 5: Continued.

Description	pvalue	qvalue	Count
PI3K-Akt signaling pathway	6.19E-12	2.53E-11	19
Lipid and atherosclerosis	7.02E-16	7.52E-14	19
Hepatocellular carcinoma	1.09E-12	6.29E-12	15
Apoptosis	1.78E-11	6.18E-11	13
p53 signaling pathway	4.76E-12	2.20E-11	11

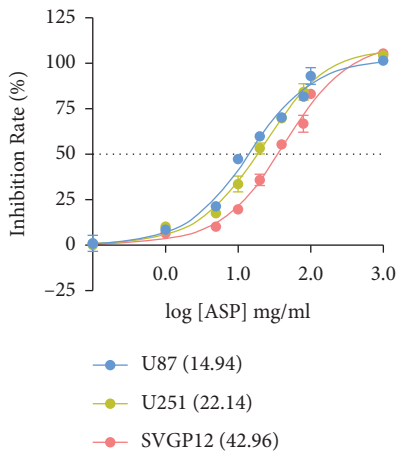


(c) (d)

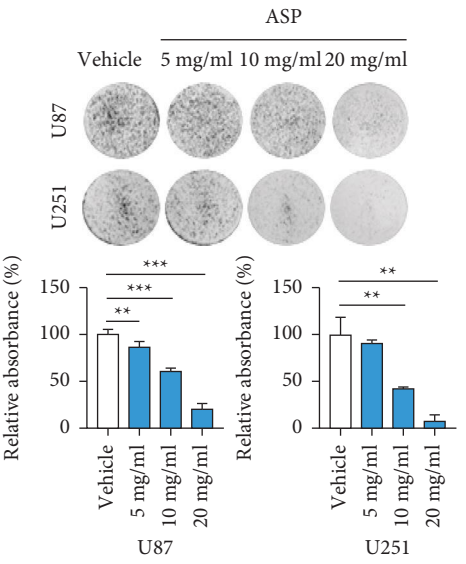


(e)

Figure 5: GO and KEGG analysis. GO function enrichment (a) and KEGG enrichment (b) bubble diagram as the result of ASP in the treatment of GBM. The color scale indicates the different thresholds of p values and the size of the dots represents the number of genes corresponding to each term. (c) Top 5 KEGG pathways. (d) Core pathways Venn diagram. The arrow indicates the hub genes. (e) The composition-target-disease-pathway network of ASP.



(a)



(b)

Figure 6: Continued.

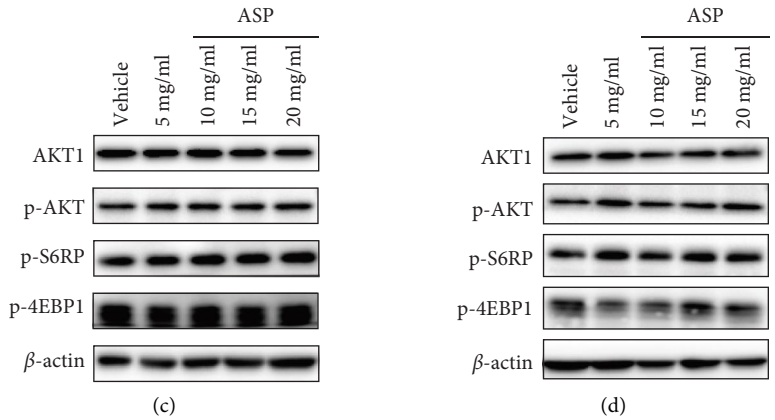


Figure 6: Inhibitory effect of ASP on GBM cells. (a) The CCK8 assay was used to determine the IC50s of SVGP12, U87, and U251 cell lines treated with ASP for 72 hours. (b) U87 and U251 cell lines were treated with ASP as indicated for 7 days and then crystal violet stained. A western blot was performed to detect the expression of AKT1, p-AKT, p-S6RP, and p-4EBP1 in ASP-treated U87 (c) and U251 (d) cells. β-actin was used as a normalization standard. The mean ± S. E. M. for three independent experiments is shown. $^{**}P < 0.01$; $^{***}P < 0.001$ (Student's t-test).

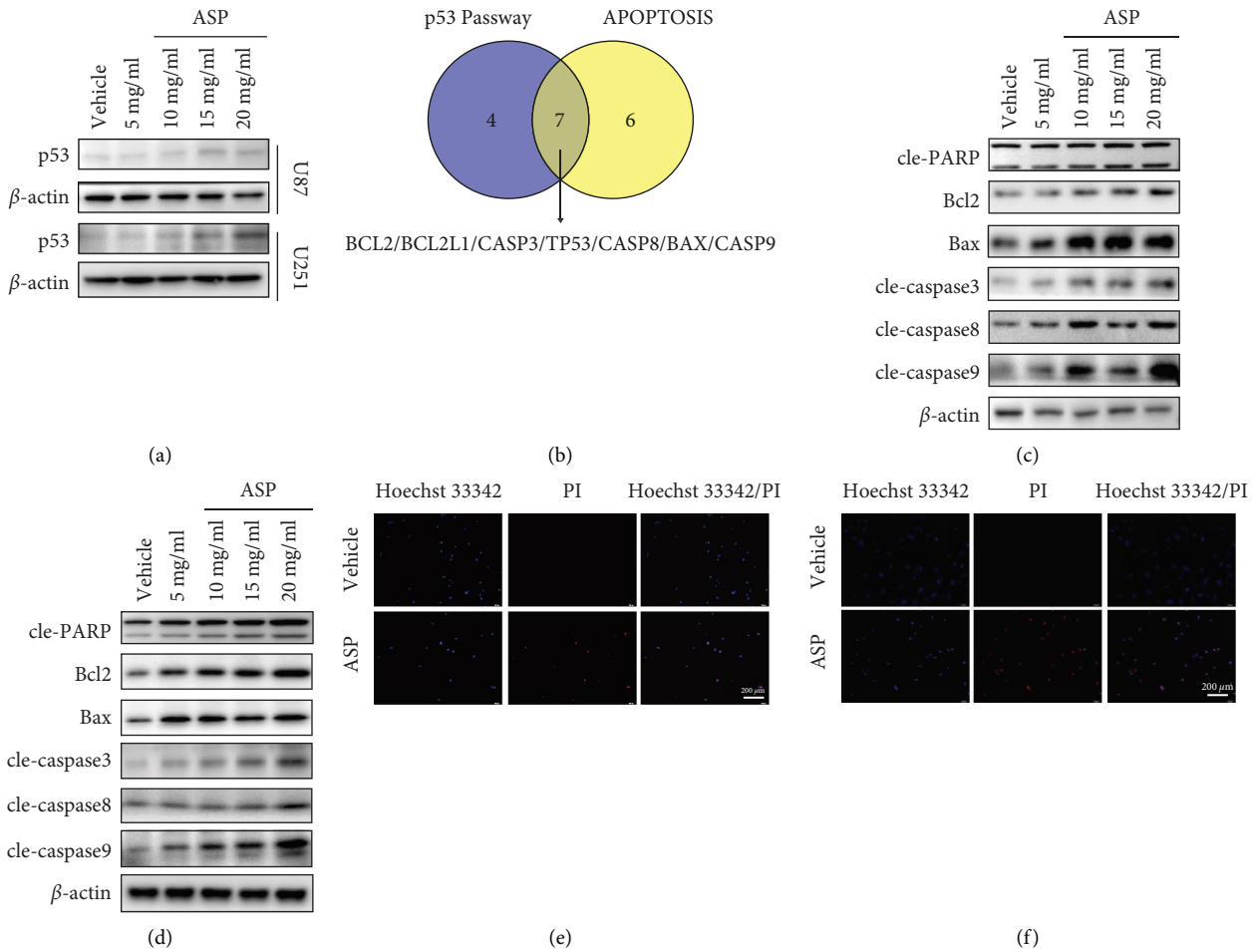
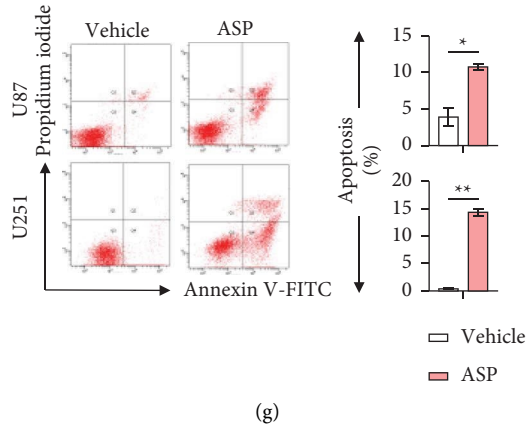


Figure 7: Continued.



(g)

Figure 7: p53-dependent apoptosis is induced by ASP activation. (a) Western blot was performed to detect the expression of P53 in ASP-treated GBM cells. (b) Pathways Venn diagram. The arrow indicates the p53-dependent apoptosis genes. Western blot was performed to detect apoptosis-related proteins in ASP-treated U87 (c) U251 (d) cells. U87 (e) and U251 (f) cells were stained with Hoechst 3342/PI to detect the effect of ASP on apoptosis. Scale bar, 200 μm. (g) A flow cytometric analysis of annexin V and propidium iodide (PI) stained cells was conducted to evaluate apoptosis. β-actin was used as a normalization standard. Mean ± S. E. M. for three independent experiments is shown. $^{*}P < 0.05$; $^{**}P < 0.01$ (Student's t-test).

occurred in response. Our study suggests that ASP can exert anticancer effects by activating P53 in GBM cells to cause p53-dependent apoptosis.

5. Conclusion

In summary, we identified the core targets of ASP for GBM treatment, AKT1, and P53, as well as 5 potential pathways through a network pharmacology approach. In vitro molecular biology studies demonstrated that ASP inhibits the proliferation of GBM cells and induces apoptosis. It was further shown that ASP induced p53-dependent apoptosis through the activation of P53 in cells rather than AKT1. In conclusion, we provide a new option for the clinical treatment of GBM patients and illustrate that network pharmacology is a powerful tool to explore the mechanism of action of traditional drugs.

Abbreviations

GBM: Glioblastoma
ASP: Asparagus
TCM: Traditional Chinese medicine
TCMSP: Traditional Chinese medicine systems pharmacology
OB: Oral bioavailability
DL: Drug-like
GO: Gene Ontology
KEGG: Kyoto Encyclopedia of Genes and Genomes
PPI: Protein-protein interaction.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

J. Y. and X. L. conceived and designed the study. J. Y. and J. X. wrote the manuscript. J. X. and X. L. performed major experiments and collected and analyzed the data. Y. G., Q. G, and M. L. provided data for partial experimental verification. All authors contributed and approved the manuscript. Xin Luo and Jinshan Xing contributed equally to this study.

Acknowledgments

This research was supported by Southwest Medical University (grant nos. 2022ZD013 and 00170031) to J. Yi.

References

[1] K. A. McNeill, "Epidemiology of brain tumors," *Neurologic Clinics*, vol. 34, no. 4, pp. 981–998, 2016.
[2] D. Krex, B. Klink, C. Hartmann et al., "Long-term survival with glioblastoma multiforme," *Brain*, vol. 130, no. 10, pp. 2596–2606, 2007.
[3] A. Schernberg, A. Marabelle, C. Massard et al., "What's next in glioblastoma treatment: tumor-targeted or immune-targeted therapies?" *Bulletin du Cancer*, vol. 103, no. 5, pp. 484–498, 2016.
[4] S. Li, Z. Wu, and W. Le, "Traditional Chinese medicine for dementia," *Alzheimer's and Dementia*, vol. 17, no. 6, pp. 1066–1071, 2021.
[5] Y. Xiang, Z. Guo, P. Zhu, J. Chen, and Y. Huang, "Traditional Chinese medicine as a cancer treatment: modern perspectives of ancient but advanced science," *Cancer Medicine*, vol. 8, no. 5, pp. 1958–1975, 2019.
[6] W. Cheng, Z. Cheng, D. Xing, and M. Zhang, "Asparagus polysaccharide suppresses the migration, invasion, and angiogenesis of hepatocellular carcinoma cells partly by targeting the HIF-1α/VEGF signalling pathway in vitro," *Evidence-based Complementary and Alternative Medicine*, vol. 2019, Article ID 3769879, 10 pages, 2019.
[7] L. Lei, Y. Chen, L. Ou, Y. Xu, and X. Yu, "Aqueous root extract of Asparagus cochinchinensis (Lour.) Merr. Has antioxidant

activity in D-galactose-induced aging mice," *BMC Complementary and Alternative Medicine*, vol. 17, no. 1, p. 469, 2017.

[8] Q. Sun, L. Zhu, Y. Li et al., "A novel inulin-type fructan from Asparagus cochinchinensis and its beneficial impact on human intestinal microbiota," *Carbohydrate Polymers*, vol. 247, Article ID 116761, 2020.

[9] L. Zhang, F. He, L. Gao et al., "Engineering exosome-like nanovesicles derived from Asparagus cochinchinensis can inhibit the proliferation of hepatocellular carcinoma cells with better safety profile," *International Journal of Nanomedicine*, vol. 16, pp. 1575–1586, 2021.

[10] T. T. Luo, Y. Lu, S. K. Yan, X. Xiao, X. L. Rong, and J. Guo, "Network pharmacology in research of Chinese medicine formula: methodology, application and prospective," *Chinese Journal of Integrative Medicine*, vol. 26, no. 1, pp. 72–80, 2020.

[11] R. Zhang, X. Zhu, H. Bai, and K. Ning, "Network pharmacology databases for traditional Chinese medicine: review and assessment," *Frontiers in Pharmacology*, vol. 10, p. 123, 2019.

[12] P. Shannon, A. Markiel, O. Ozier et al., "Cytoscape: a software environment for integrated models of biomolecular interaction networks," *Genome Research*, vol. 13, no. 11, pp. 2498–2504, 2003.

[13] D. Szklarczyk, A. L. Gable, D. Lyon et al., "STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets," *Nucleic Acids Research*, vol. 47, no. D1, pp. D607–d613, 2019.

[14] H. M. Berman, J. Westbrook, Z. Feng et al., "The protein data bank," *Nucleic Acids Research*, vol. 28, no. 1, pp. 235–242, 2000.

[15] D. Seeliger and B. L. De Groot, "Ligand docking and binding site analysis with PyMOL and Autodock/Vina," *Journal of Computer-Aided Molecular Design*, vol. 24, no. 5, pp. 417–422, 2010.

[16] O. Trott and A. J. Olson, "AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading," *Journal of Computational Chemistry*, vol. 31, no. 2, pp. 455–461, 2010.

[17] H. Gong, Y. Cao, G. Han et al., "p53/microRNA-374b/AKT1 regulates colorectal cancer cell apoptosis in response to DNA damage," *International Journal of Oncology*, vol. 50, no. 5, pp. 1785–1791, 2017.

[18] S. Y. Lee, "Temozolomide resistance in glioblastoma multiforme," *Genes & Diseases*, vol. 3, no. 3, pp. 198–210, 2016.

[19] X. Wang, Z. Y. Wang, J. H. Zheng, and S. Li, "TCM network pharmacology: a new trend towards combining computational, experimental and clinical approaches," *Chinese Journal of Natural Medicines*, vol. 19, no. 1, pp. 1–11, 2021.

[20] H. Xiao, Z. Deng, J. T. Hough et al., "The effect of Asparagus extract on pancreatic cancer: an intriguing surprise," *Anticancer Research*, vol. 42, no. 5, pp. 2425–2432, 2022.

[21] A. Romani, F. Casciano, C. Stevanin et al., "Anticancer activity of aqueous extracts from asparagus officinalis l. byproduct on breast cancer cells," *Molecules*, vol. 26, no. 21, p. 6369, 2021.

[22] S. Bousserouel, J. Le Grandois, F. Gossé et al., "Methanolic extract of white asparagus shoots activates TRAIL apoptotic death pathway in human cancer cells and inhibits colon carcinogenesis in a preclinical model," *International Journal of Oncology*, vol. 43, no. 2, pp. 394–404, 2013.

[23] A. L. Hopkins, "Network pharmacology: the next paradigm in drug discovery," *Nature Chemical Biology*, vol. 4, no. 11, pp. 682–690, 2008.

[24] Y. Li, J. Yao, C. Han et al., "Quercetin, inflammation and immunity," *Nutrients*, vol. 8, no. 3, p. 167, 2016.

[25] H. I. Kim, S. J. Lee, Y. J. Choi, M. J. Kim, T. Y. Kim, and S. G. Ko, "Quercetin induces apoptosis in glioblastoma cells by suppressing axl/IL-6/STAT3 signaling pathway," *American Journal of Chinese Medicine*, vol. 49, no. 03, pp. 767–784, 2021.

[26] L. Wang, S. Ji, Z. Liu, and J. Zhao, "Quercetin inhibits glioblastoma growth and prolongs survival rate through inhibiting glycolytic metabolism," *Chemotherapy*, vol. 67, no. 3, pp. 132–141, 2022.