




Article

A Network-Based Approach to Explore the Mechanisms of *Uncaria* Alkaloids in Treating Hypertension and Alleviating Alzheimer's Disease

Wenyong Wu ^{1,2,†}, Zijia Zhang ^{1,†}, Feifei Li ^{1,2}, Yanping Deng ¹, Min Lei ¹ , Huali Long ¹, Jinjun Hou ^{1,*} and Wanying Wu ^{1,*}

¹ Center for Modernization of Traditional Chinese Medicine, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 501 Haik Road, Pudong New District, Shanghai 201203, China; 2017e8012363117@simm.ac.cn (W.W.); zijiazhang@simm.ac.cn (Z.Z.); s19-lifeifei@simm.ac.cn (F.L.); shimbiro@163.com (Y.D.); mlei@simm.ac.cn (M.L.); longhuali@simm.ac.cn (H.L.)

² University of Chinese Academy of Sciences, No. 19A Yuquan Road, Beijing 100049, China

* Correspondence: jinjun_hou@simm.ac.cn (J.H.); wanyingwu@simm.ac.cn (W.W.); Tel.: +86-021-5080-2351 (J.H.)

† These authors contributed equally to this work.

Received: 3 February 2020; Accepted: 2 March 2020; Published: 4 March 2020



Abstract: *Uncaria* alkaloids are the major bioactive chemicals found in the *Uncaria* genus, which have a long history of clinical application in treating cardiovascular and mental diseases in traditional Chinese medicine (TCM). However, there are gaps in understanding the multiple targets, pathways, and biological activities of *Uncaria* alkaloids. By constructing the interactions among drug-targets-diseases, network pharmacology provides a systemic methodology and a novel perspective to present the intricate connections among drugs, potential targets, and related pathways. It is a valuable tool for studying TCM drugs with multiple indications, and how these multi-indication drugs are affected by complex interactions in the biological system. To better understand the mechanisms and targets of *Uncaria* alkaloids, we built an integrated analytical platform based on network pharmacology, including target prediction, protein–protein interaction (PPI) network, topology analysis, gene enrichment analysis, and molecular docking. Using this platform, we revealed the underlying mechanisms of *Uncaria* alkaloids' anti-hypertensive effects and explored the possible application of *Uncaria* alkaloids in preventing Alzheimer's disease. These results were further evaluated and refined using biological experiments. Our study provides a novel strategy for understanding the holistic pharmacology of TCM, as well as for exploring the multi-indication properties of TCM beyond its traditional applications.

Keywords: *Uncaria* alkaloids; network pharmacology; hypertension; Alzheimer's disease; butyrylcholinesterase; molecular docking

1. Introduction

The *Uncaria* stem with hooks (as known as gou-teng in Chinese) is widely used clinically as a formulae medicine with anti-hypertensive effects in traditional Chinese medicine (TCM) [1–3]. As the main bioactive compounds in the *Uncaria* plants, *Uncaria* alkaloids have shown significant hypotensive potency in the spontaneously hypertensive rat model [1,4]. Besides the effects on the cardiovascular system, *Uncaria* plants are also documented in ancient TCM formulae that alleviate symptoms associated with the central nervous system (CNS), such as febrile seizures, dizziness, and migraines [1–3]. A few recent studies show that rhynchophylline, one of the most widely studied indole alkaloids from the *Uncaria* plants, has effects in reducing aggregation and deposition of amyloid

proteins and inhibiting extrasynaptic *N*-methyl-D-aspartate (NMDA) receptor activation [1,2,5–10]. These studies indicate that the bioactive molecules extracted from the *Uncaria* plants may have potential in treating neurodegenerative diseases like Alzheimer's disease (AD).

Currently, there is no cure available in reversing AD disease progression, and most approved drugs for AD treatment are for symptom alleviation, e.g., improving mental functions, reducing behavioral problems from AD, or slowing down memory decline [11–13]. Much effort has been made to search for the genetic and environmental factors contributing to the pathogenesis of AD among the aging population. Vascular damage, including capillary rarefaction, blood–brain barrier disruption, and consequential neuroinflammation, impairs neurovascular-coupling responses and promotes the genesis of cerebral microhemorrhages [14,15]. It explains many of the biological processes and pathogenesis of AD and this is supported by many epidemiological and experimental studies [14,16–22].

Impaired CNS vasculature causes permanent damage to hippocampal formation and cognitive functions [23]. It indicates that drugs used in hypertension treatment may have additional benefits in slowing down AD disease progression. Based on this hypothesis, studies and clinical trials have been carried out to evaluate nilvadipine, a calcium channel blocker for the treatment of hypertension, for delaying AD disease progression [24,25]. Despite nilvadipine's conflicting clinical study results in treating AD, it is still accepted that hypertension promotes the pathogenesis of AD, and reducing hypertension before or during AD onset may help slow down AD disease progression [24–27].

The discovery of artemisinin is a phenomenal example to demonstrate that traditional Chinese medicine (TCM) is an invaluable source for the modernization of conventional drugs [28,29]. Much effort has been made to understand the complex system of TCM, with a combination of its traditional treatments and modern technological means [30–32]. Network pharmacology is a branch of system biology that facilitates drug identification and design for multi-target compounds by selecting the key genes and their biological processes and functional pathways. It integrates and visualizes the complex networks among compounds, targets, disease targets, and biological processes [33]. With the help of network pharmacology, it is possible to reveal the intricate molecular mechanisms underlying TCM and further establish novel therapeutic application beyond the traditional TCM application. For example, in combination with a strategy using high-throughput sequencing technique, Li S. et al. screened 22 health-strengthening and 25 pathogen-eliminating herbs to identify potential target profiles of 1446 TCM compounds, and several cancer-related pathways of those compounds were identified, such as apoptosis, vascular endothelial growth factor (VEGF) signaling pathway, T cell receptor related pathways, etc. Those herbs have a more significant regulatory role in immune and tumor microenvironment, rather than direct cytotoxic effects on tumors [34]. As for exploring novel TCM applications using network pharmacology, Li JY et al. combined network pharmacology, machine learning, deep learning, and molecular dynamics simulation to investigate potent lead drugs to identify insulin-like growth factor 1 receptor (IGF1R) and insulin receptor (IR) as two key genes, as well as three potential longevity-related herbal drugs from the established TCM databases [35]. It is known that IGF1R and IR are related to tumor growth and insulin insensitivity, respectively. These results indicate that the three longevity-related herbs have novel therapeutic potentials in cancer treatment and type II diabetes in a novel or improved TCM formulae. The above examples show that studies on this scale are impossible to perform using traditional laboratory techniques and resources, but that network pharmacology makes it possible and illuminates directions for future studies. Therefore, network pharmacology can help optimize research design, establishing novel clinical applications, and improving cost efficiency, which will be beneficial to the research and advance of TCM.

In this study, we use network pharmacology to screen the *Uncaria* alkaloids that may alleviate AD symptoms via working on the cardiovascular system and also to find their potential targets and molecular mechanisms. To better understand the mechanisms and targets of these alkaloids, the in-house library of *Uncaria* alkaloids was compiled from our previous study. An integrated analytical platform was built based on network pharmacology, including target prediction, protein–protein interaction (PPI) network, topology analysis, gene enrichment analysis, and molecular docking.

By combining this platform and biological experiments, we explored the underlying mechanisms of *Uncaria* alkaloids' anti-hypertensive effects and explored the possible application of *Uncaria* alkaloids in preventing AD.

2. Results

The schematic workflow of this study is demonstrated in the graphical abstract.

2.1. The In-House Library of *Uncaria* Alkaloids and Drug-Likeness Screening

One hundred and forty-three *Uncaria* alkaloids from *Uncaria* genus were included in the in-house compound library for drug-likeness analysis using the FAFDrugs4 program. From 143 compounds, 83 were accepted as candidate compounds (Table S1). Out of the rejected compounds, 3-dihydrocadambine (546.6 g/mol) and vincosamide (498.5 g/mol) were rejected because their predicted numbers of rigid bonds and max ring size exceeded the threshold. They were included in the library based on their reported bioactivities from the literature search or previous studies [4,36,37]. Therefore, a total of 85 *Uncaria* alkaloids were used in the following target prediction.

2.2. Putative Target Prediction for the Candidate Targets of *Uncaria* Alkaloids

To find the target genes of the 85 *Uncaria* alkaloids, PhamaMapper, TargetNet, SEA and SwissTarget servers were used to predict the *Uncaria* alkaloids' compound targets, and the numbers of overlapped targets were visualized in Figure 1a. There were 103 targets from PharmMapper (Norm fit > 0.8), 78 targets from TargetNet server (probability > 0.8), 89 targets identified in SEA database, and 16 from SwissTarget (probability > 0.5) (Figure 1b). No shared targets were found at the intersection of all four databases. Overall, there are 227 specific genes, 22 genes shared by two databases, and only five genes found in three databases (Figure 1c). After removal of the partially overlapped targets, 254 genes were found in the union, and they were selected as the potential targets of *Uncaria* alkaloid for the following analysis. The 254 genes and their functional annotations were visualized using ClueGO software (Figure S1).

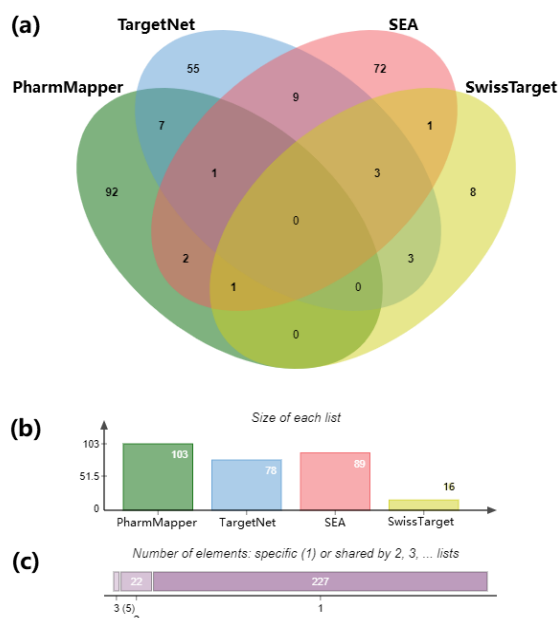


Figure 1. Analysis of the predicted genes for the 85 *Uncaria* alkaloids from four databases. (a) Venn graph showing the numbers of the overlapped and the specific genes predicted from PharmMapper (green), TargetNet (blue), SEA (pink), and SwissTarget (yellow); (b) numbers of predicted genes from each database; (c) a summary of numbers of genes shared among the databases: 227 specific genes, 22 genes shared by two databases, and five genes found in three databases.

2.3. Identification of Approved Druggable Targets in AD and Hypertension

DrugBank and TTD databases were used to identify the approved therapeutic targets of AD and hypertension, and the overlap of the results was visualized in the venn graph (Figure 2a). After redundancy removal, 102 therapeutic targets were identified for AD and 181 for hypertension (Figure 2b). When overlapped with the 254 targets of *Uncaria* alkaloids, twenty-three targets were found in the intersection of *Uncaria* alkaloids, AD, and hypertension (Figure 2a).

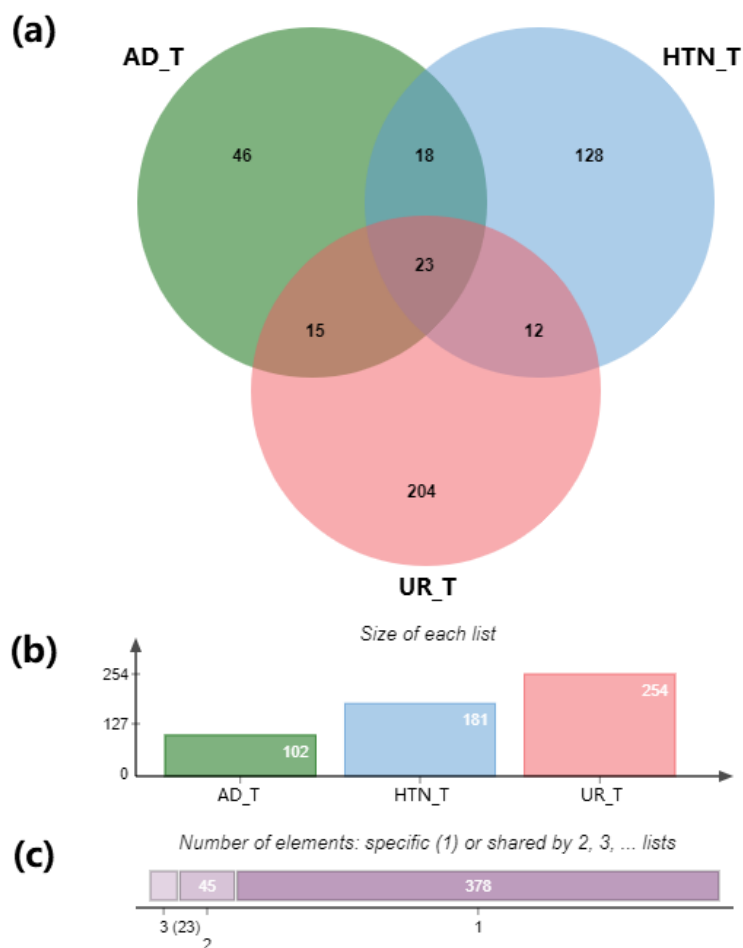


Figure 2. Analysis of the predicted compound-disease target genes among the 85 *Uncaria* alkaloids (UR_T), Alzheimer’s disease (AD_T), and hypertension (HTN_T). **(a)** Venn graph showing the numbers of the overlapped and the specific genes among AD, HTN, and UR; **(b)** numbers of predicted genes from each set; **(c)** a summary of numbers of genes shared among the gene sets: 378 specific genes, 45 genes shared by two sets, and 23 genes found in all three sets of genes.

2.4. Pathways in Compound-Target Network

To verify the shared targets among *Uncaria* alkaloids (UR) and the disease targets from Alzheimer’s disease (AD) and hypertension (HTN), the 38 shared targets in “AD_UR” and the 35 shared targets in “HTN_UR” were analyzed using MetaScape (Figures 2a and 3). The heatmap of GO analysis showed that “amine ligand-binding receptors”, “response to xenobiotic stimulus”, “blood circulation”, and “G protein-coupled serotonin receptor signaling pathway” are important biological processes shared by AD_UR and HTN_UR (Figure 3).

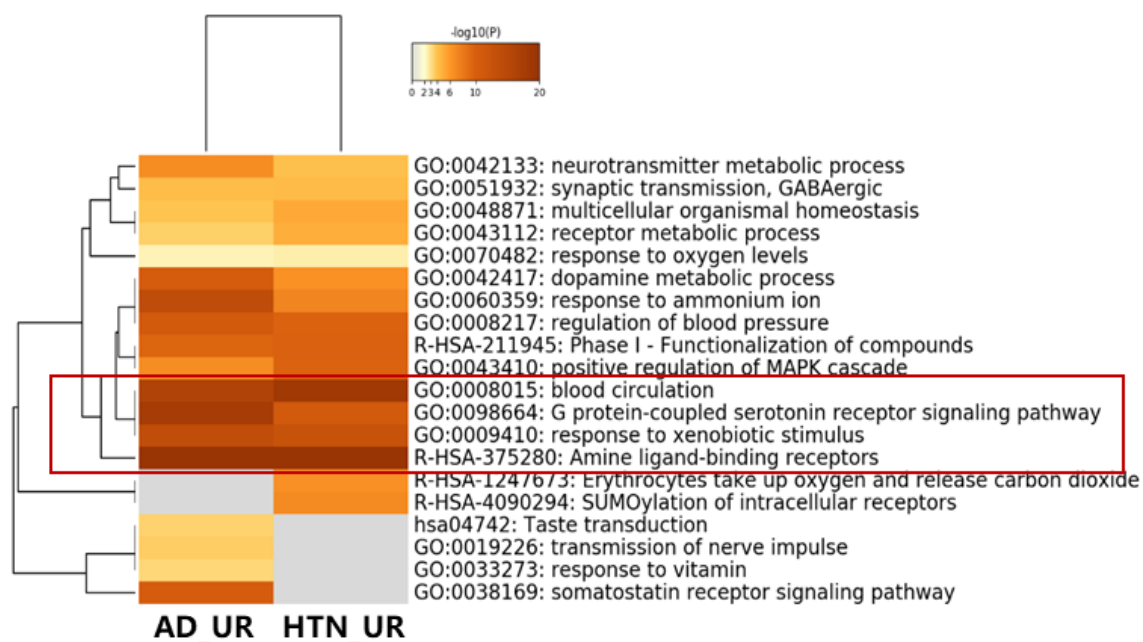


Figure 3. Heatmap of pathway analysis between AD_UR and HTN_UR using Metascape database. Targets in AD_UR and HTN_UR shared important biological processes, such as “amine ligand-binding receptors”, “response to xenobiotic stimulus”, “blood circulation”, and “G protein-coupled serotonin receptor signaling pathway”.

2.5. Network Construction, Topological Analysis, and GO/KEGG Function and Pathway Analysis

All genes were presented in HGNC (HUGO Gene Nomenclature Committee) gene symbol format in the following analysis to avoid confusion across databases and platforms. The interaction between the 23 genes was analyzed and visualized using STRING and Cytoscape databases. Under “medium confidence (0.4 by default)”, the protein–protein interaction (PPI) network was constructed (Figure 4). The results and parameters from the PPI topological analysis are listed in Table 1. MAOA (monoamine oxidase A), ACHE (acetylcholinesterase), BCHE (butyrylcholinesterase), DRD2 (dopamine receptor D2), and HTR1A (serotonin 1A receptor) were the top 5 genes based on degree (Table 1, Column 3). The scores of other parameters are listed in Table 1 (Columns 4–10)—no significant alterations were found when the target ranks were based on other parameters (Table 1).

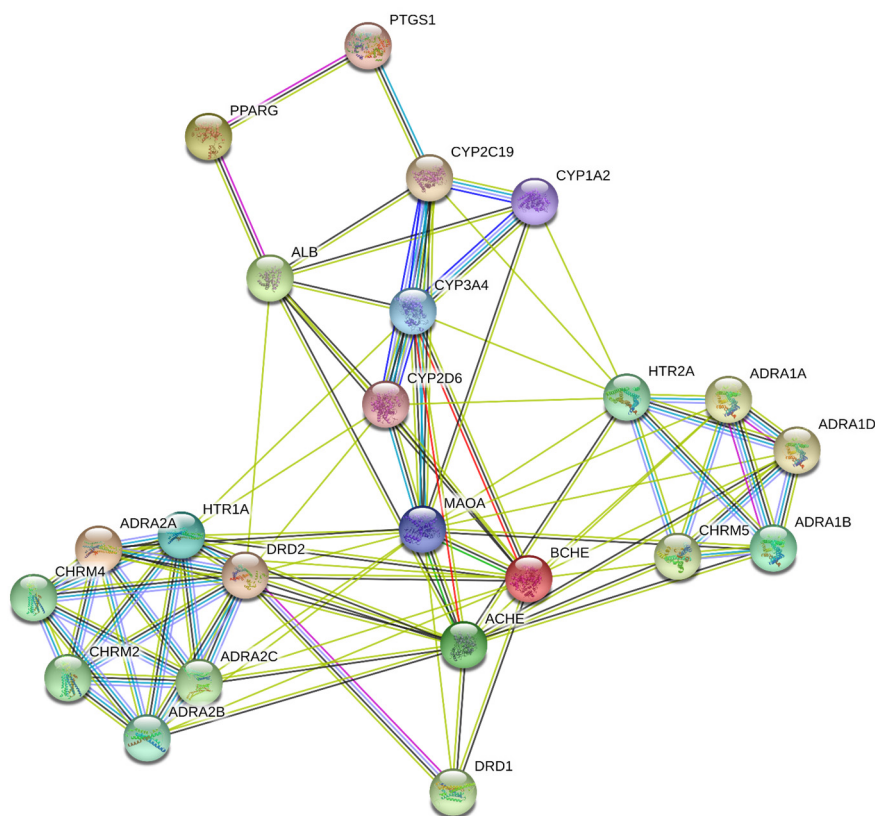


Figure 4. Visualization of the protein–protein interaction (PPI) of the 23 target genes* using STRING and Cytoscape databases.

Table 1. Topological analysis of the PPI network of the 23 genes shared by *Uncaria* alkaloids, AD and hypertension. Data were ranked by degree.

#	Gene Symbol	Degree	Subgraph	Eigenvector	Information	LAC	Betweenness	Closeness	Network
1	MAOA	16	1850.135	0.356	5.360	6.375	81.651	0.759	13.078
2	ACHE	14	1413.283	0.311	5.162	5.143	63.508	0.710	9.577
3	BCHE	13	1311.436	0.300	5.048	4.923	38.836	0.688	8.028
4	DRD2	12	1230.716	0.290	4.924	6.333	35.935	0.667	9.991
5	HTR1A	11	1166.267	0.282	4.786	6.545	20.340	0.629	9.071
6	HTR2A	10	623.717	0.203	4.632	4.800	24.033	0.595	7.526
7	ADRA2B	9	868.194	0.242	4.461	6.444	7.798	0.595	8.000
8	ADRA2C	9	868.194	0.242	4.461	6.444	7.798	0.595	8.000
9	ADRA2A	9	868.194	0.242	4.461	6.444	7.798	0.595	8.000
10	CYP3A4	9	688.133	0.216	4.461	4.889	10.826	0.629	6.405
11	ALB	8	406.076	0.165	4.268	2.750	47.899	0.611	3.429
12	CYP2D6	8	606.406	0.203	4.268	4.500	9.607	0.611	5.238
13	ADRA1D	7	428.160	0.169	4.049	4.857	1.567	0.537	6.000
14	ADRA1B	7	428.160	0.169	4.049	4.857	1.567	0.537	6.000
15	ADRA1A	7	428.160	0.169	4.049	4.857	1.567	0.537	6.000
16	CYP2C19	7	271.666	0.133	4.049	3.429	39.552	0.564	4.333
17	CHRM2	6	348.284	0.151	3.798	5.000	0.000	0.458	6.000
18	CHRM4	6	348.284	0.151	3.798	5.000	0.000	0.458	6.000
19	CHRM5	5	178.487	0.107	3.508	4.000	0.000	0.512	5.000
20	CYP1A2	5	193.747	0.112	3.508	3.200	1.269	0.537	4.000
21	DRD1	4	252.070	0.131	3.170	2.500	0.167	0.524	3.333
22	PPARG	2	7.872	0.019	2.287	0.000	2.700	0.400	0.000
23	PTGS1	2	6.536	0.016	2.287	0.000	1.583	0.379	0.000

2.6. Pathways in Target-Disease Network

The list of the 23 screened genes was uploaded to the WebGestalt database for GO enrichment analysis. Three categories, i.e., biological process (BP), cellular component (CC), and molecular function

(MF), are presented in Figure 5. The 23 targets were involved in many biological processes including “response to stimulus”, “biological regulation”, “metabolic process”, and “cell communication” (Figure 5a). “Membrane” and “endomembrane system” ranked the highest in the cellular component category (Figure 5b), while “protein binding”, “molecular transducer activity”, and “ion binding” were the primary molecular function involved (Figure 5c).

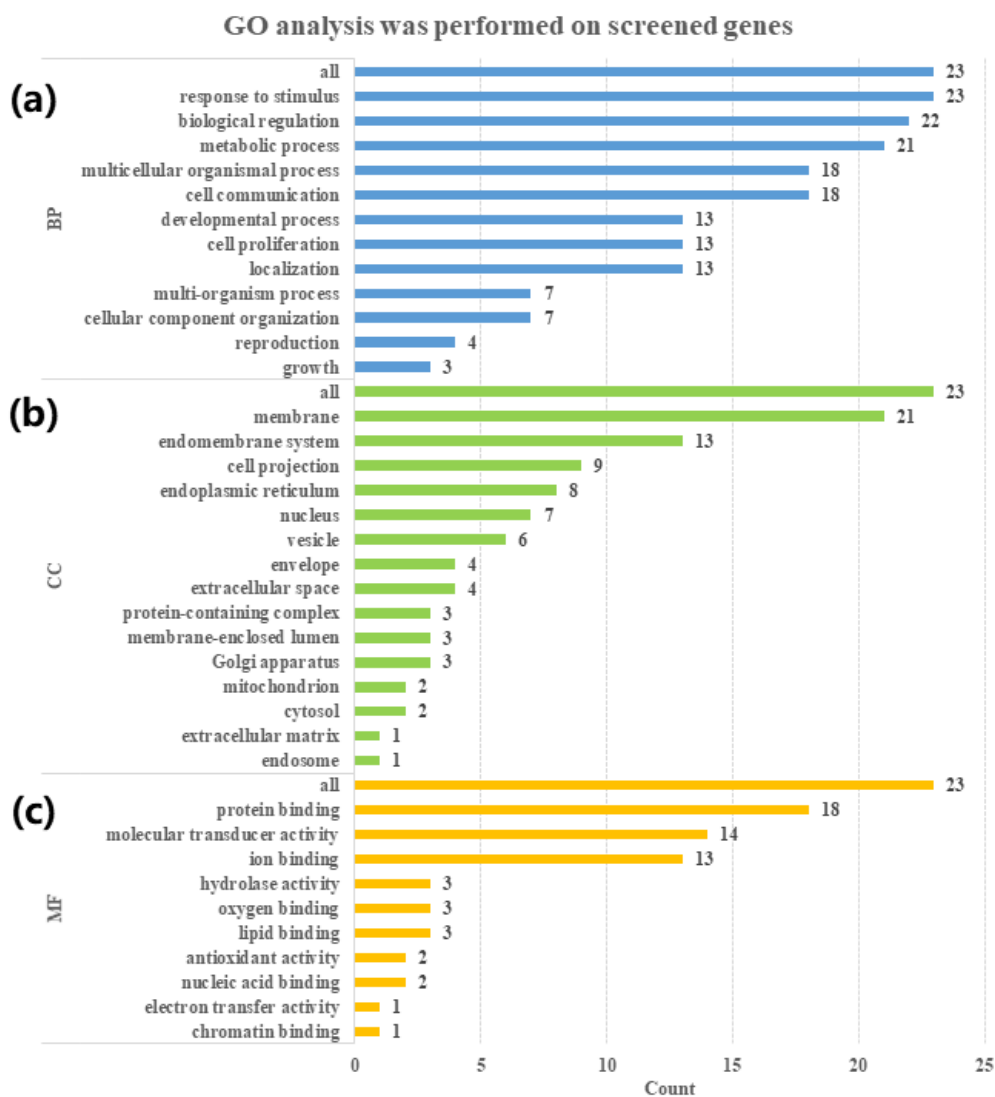


Figure 5. GO analysis of target–disease gene interactions for *Uncaria* alkaloids to reveal the related (a) biological process (BP); (b) cellular component (CC); and (c) molecular function (MF). The numbers of genes included are marked next to the horizontal bar graph.

2.7. Pathways in Target-Pathway Network

Using the ConsensusPathDB-human database, the top 20 pathways of the 23 predicted genes are listed in Figure 6—the two pathways that showed statistical significance are “monoamine GPCRs” and “amine ligand-binding receptors” and are represented by the two red dots in the bubble graph (Figure 6). There were 13 out of the 23 screened genes present in the “monoamine GPCRs” pathway. More detailed pathway information of the 13 genes is highlighted in Figure 7, showing that they are distributed in categories of adrenergic receptors, dopamine receptors, serotonin receptors, and muscarinic acetylcholine receptors, but not in histamine receptors (Figure 7).

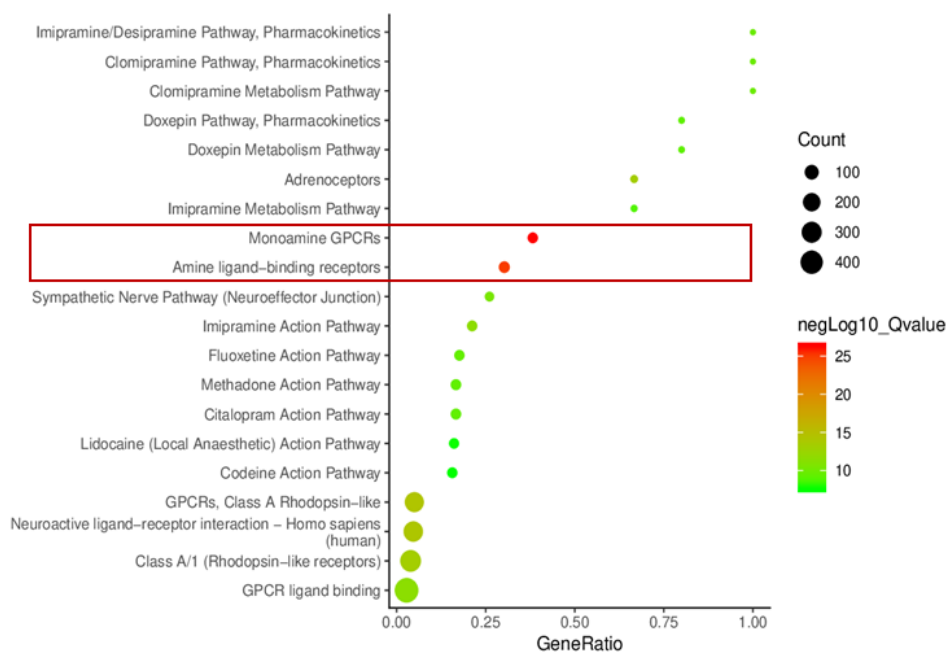


Figure 6. Bubble graph demonstrating statistically significant pathways that include the 23 genes analyzed using the ConsensusPathDB-human database. The results showed that “amine ligand-binding receptors” and “monoamine GPCRs” are two important pathways regarding *Uncaria* alkaloids, AD, and hypertension.

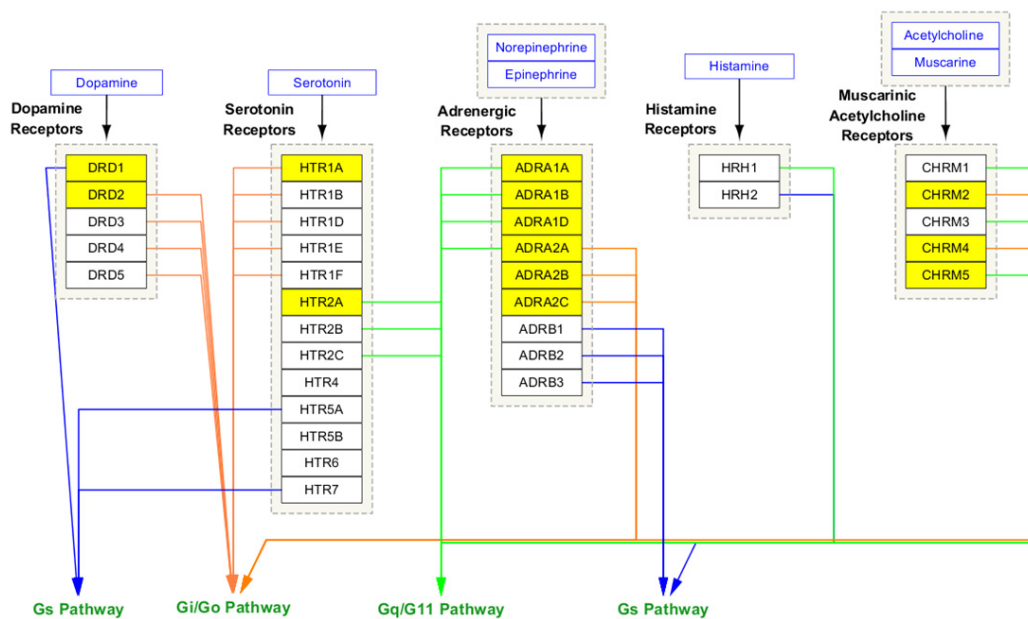


Figure 7. Detailed analysis of the 13 out of the 23 screened genes in “monoamine GPCRs” pathway. The 13 genes are highlighted, and they are distributed in categories of adrenergic receptors, dopamine receptors, serotonin receptors, and muscarinic acetylcholine receptors, but not in histamine receptors. Arrows: blue, Gs pathway; orange, Gi/Go pathway; green, Gq/G11 pathway.

2.8. Integrative Network Analysis and Target Selection

To further obtain a comprehensive understanding between the selected *Uncaria* alkaloids, the selected target genes, and the diseases, an integrative network analysis was performed using Cytoscape (Figure 8). An intricate network was formed among the *Uncaria* alkaloids and their potential targets regarding AD and hypertension.

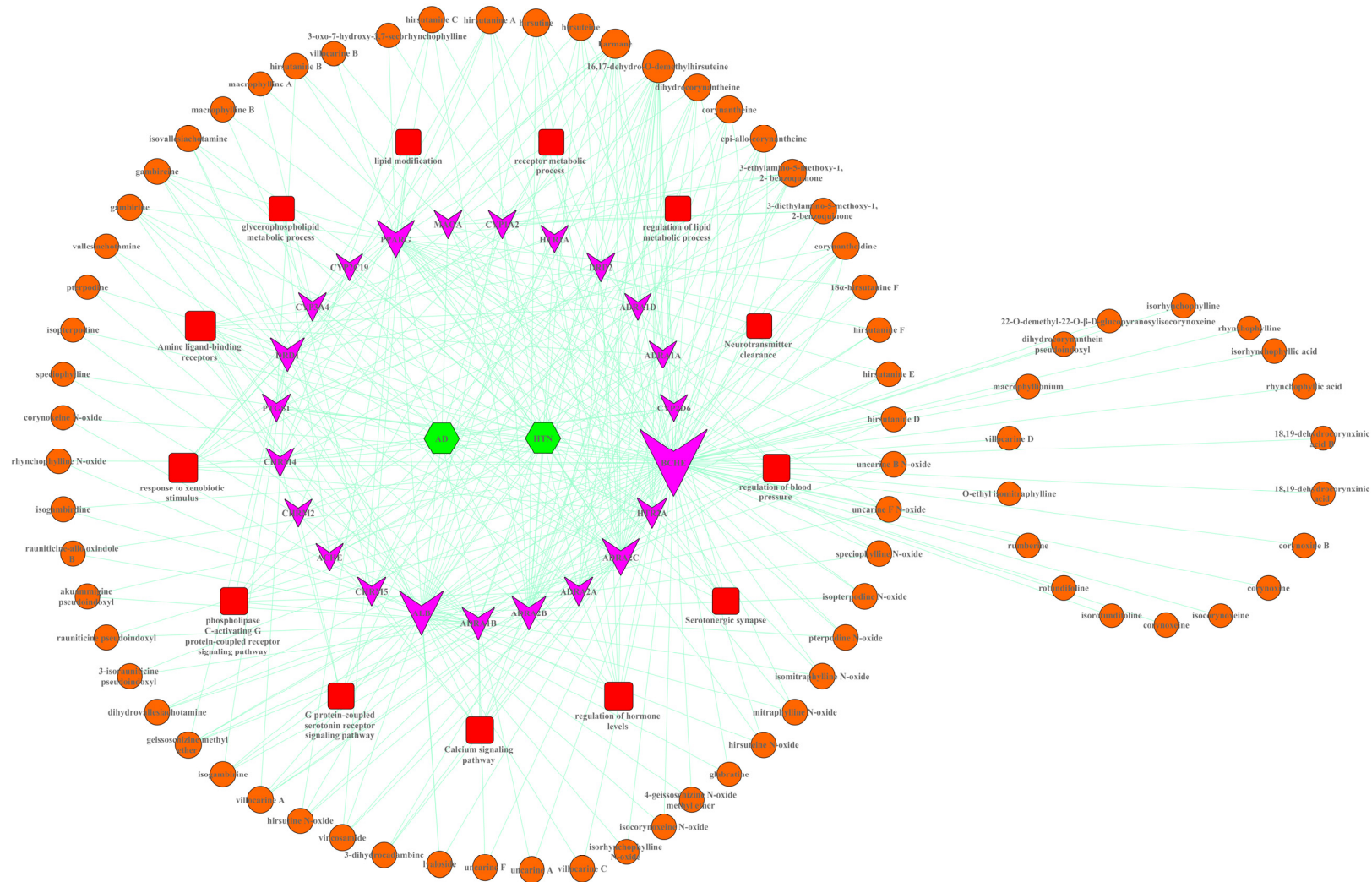


Figure 8. Comprehensive representation of the built network of *Uncaria* alkaloids, AD, and hypertension. Diseases are represented by hexagons (green), target genes by arrowheads (magenta), *Uncaria* alkaloids by circles (orange), and pathways by squares (red).

2.9. Molecular Docking and Biological Validation

Among the top six genes in Table 1, there were 74 *Uncaria* alkaloids identified for BCHE. Only four *Uncaria* alkaloids were corresponding to the other five genes (MAOA, ACHE, DRD2, HTR1A, and HTR2A) across various databases. Therefore, BCHE was chosen as a more efficient target for the following biological validation (see Raw data.elsx). To validate the ability of *Uncaria* alkaloids to target the selected genes, we chose BChE (PDB ID: 4TPK) as an example for molecular docking. The scores are listed in Table 2. As shown, hirsuteine (HTE) and hirsutine (HTI) were the two compounds with the highest scores of 6.7146 and 6.4769, respectively (Table 2). Their binding sites and affinity (2D ligand–protein interaction diagrams) are also shown in Figure 9. Galantamine hydrobromide, a selective AChE inhibitor, had a lower score of 4.8609, while the BChE inhibitor drofenine hydrochloride had the highest score of 9.7639.

The biological effects of the selected *Uncaria* alkaloids were further evaluated using BChE enzyme activity assay, with HTE and HTI showing significant inhibitory effects on BChE activity. The IC_{50} of each compound was 67.97 μ M and 202.3 μ M (Table 3 and Figure 10). Geissoschiziner, corynoxine and isocorynoxine showed moderate inhibitory effects on BChE at a high concentration of 500 μ M (Table 3). No inhibitory activity was detected in rhynchophylline, isorhynchophylline, 3-dihydrocadambine, or vincosamide (data not shown).

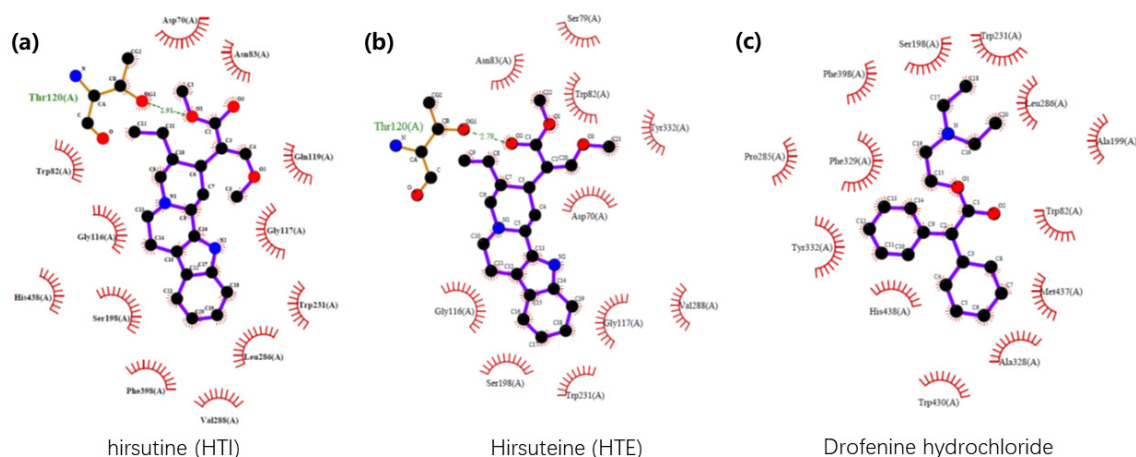


Figure 9. Schematic diagrams demonstrating the butyrylcholinesterase (BChE) binding sites and proximate affinity of hirsutine (HTI), hirsuteine (HTE) and drofenine hydrochloride (a BChE inhibitor). Black dots: carbon atoms; blue dots: nitrogen atoms; red dots: oxygen atoms; green dotted lines: hydrogen bonds; red combs: amino acid residues.

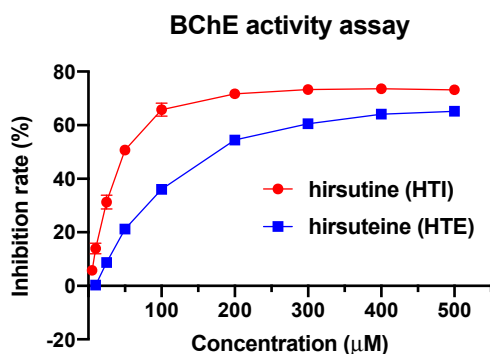


Figure 10. Inhibition rate of hirsutine (HTI) and hirsuteine (HTE) in BChE activity assay. HTI IC_{50} = 67.97 μ M; HTE IC_{50} = 202.3 μ M.

Table 2. Selected *Uncaria* alkaloids and their total scores from molecular docking analysis using SYBYL-X 2.0 Software.

Ligand	Total Scores
3F9_A	5.524
3F9_B	4.6765
Corynoxine B	5.7701
Corynoxine (C)	5.4715
Corynoxine	5.58
3-dihydrocadambine (DHC)	5.0741
Geissoschiziner (GSM)	5.886
Hirsuteine (HTE)	6.7146
Hirsutine (HTI)	6.4769
Isocorynoxine (IC)	6.196
Isorhynchophylline (IR)	6.1038
isomitraphylline	4.3782
Rhynchophylline (R)	4.8015
Uncarine B	4.8631
Vincosamide (VCA)	5.9499
Galantamine hydrobromide	4.8609
Drofenine hydrochloride	9.7639

Table 3. In vitro BChE enzyme activity assay of five selected *Uncaria* alkaloids.

Conc. (μ M)	Inhibition Rate (%)				
	Hirsutine (HTI)	Hirsuteine (HTE)	Geissoschiziner (GSM)	Corynoxine	Isocorynoxine
50	50.71 \pm 1.51	21.23 \pm 0.74	0.07 \pm 0.99	-	-
100	65.83 \pm 2.49	36.03 \pm 0.67	7.34 \pm 0.66	-	-
200	71.69 \pm 0.18	54.44 \pm 0.92	22.24 \pm 1.32	(-1.86) \pm 1.65	(-1.86) \pm 0.55
300	73.28 \pm 0.67	60.51 \pm 0.74	n.a.	1.76 \pm 0.98	3.09 \pm 1.58
400	73.60 \pm 0.67	64.13 \pm 0.18	n.a.	16.82 \pm 3.39	15.17 \pm 3.50
500	73.17 \pm 1.15	65.19 \pm 0.96	39.83 \pm 1.52	19.43 \pm 3.21	21.77 \pm 0.90
IC ₅₀	67.97	202.3	n.a.	n.a.	n.a.

3. Discussion

As one of the representative herbs of traditional Chinese medicine (TCM), the *Uncaria* genus has received extensive investigation in modern pharmacology due to its effects on alleviating symptoms related to the cardiovascular system and central nervous system (CNS) [1–3,5,7]. To better understand the mechanisms behind the clinical applications of *Uncaria*, we built an integrated analytical platform based on network pharmacology, including target prediction, protein–protein interaction (PPI) network, topology analysis, gene enrichment analysis, and molecular docking. Using this platform, we identified some underlying mechanisms of anti-hypertensive effects and explored the possibility of *Uncaria* alkaloids in preventing Alzheimer’s disease (AD). The butyrylcholinesterase (BChE) enzyme activity assay provided further supports for the results from the network analysis and molecular docking, showing the feasibility of the current analytical approach. Therefore, using *Uncaria* as an example, this study provides a novel strategy for understanding the holistic pharmacology of TCM, as well as for exploring the multi-indication property of TCM beyond its traditional applications.

Uncaria genus can be found in many ancient TCM formulas treating hypertension, and *Uncaria* alkaloids are considered as the major bioactive compounds [1,4,5]. For example, rhynchophylline and isorhynchophylline, two of the most widely studied indole alkaloids from the *Uncaria* genus, can inhibit the neuronal release of catecholamines, block the conduction between neurons, and indirectly achieve anti-hypertensive effects [7,38–41]. Further evidence indicates that the cardiovascular regulation of rhynchophylline and isorhynchophylline comes from direct vasodilation, down-regulation of cardiac

output, and reduction of calcium influx [1,7,42]. All these actions cause vasodilation and lead to the reduction of blood pressure, yet the exact mechanisms are still to be elucidated.

Like in other systems of the human body, vascular impairment in the brain causes further tissue and organ damage, usually in the form of chronic neuroinflammation, which can lead to neurodegenerative diseases [15–18,20,21]. Though age and genetics contribute a significant part to AD pathogenesis, hypertension is also generally accepted as one of the significant risk factors for AD, together with other risk factors including diabetes mellitus, head trauma, and depression [17,43–45]. Ye et al. revealed that rhynchophylline has therapeutic potentials in alleviating AD via targeting the EphA4 receptor [46]. Other studies on *Uncaria* and AD have shown that *Uncaria* alkaloids have neuroprotective effects on reducing beta-amyloid aggregation and deposition [6,8,47]. From the PPI network and topological analysis in our study, several possibly synergic and promising effects of *Uncaria* have been revealed for the slowing down, if not blocking or reversing, of AD pathogenesis (Figure 5 and Table 1). The “multiple mechanisms and multitargets” property of *Uncaria*’s effects on AD is also no exception to the characteristic pharmacological effects of natural products, which often demand a more comprehensive and integrative analytical strategy to study.

In 2002, Dr. Shao Li and his team elaborated on the non-linear, open, and complex properties of TCM from a symptomatological, network, and systemic perspective [30,31,48]. British pharmacologist Dr. Andrew L. Hopkins established the concept of “network pharmacology”, and defined it as a subdivision of pharmacology, which can analyze the overall effects and interactions among the multiple compounds, their targets, and the related pathways [49,50]. With the help of network pharmacology, significant progress has been made in understanding the complicated and complex systems such as the pharmacology of TCM formula and compositions [34,51–54].

During the analytical workflow of network pharmacology, the critical step is the prediction and selection of the targets. Currently, two common approaches are available, one based on the ligands and the other based on the receptors for drug design, drug discovery and evaluation [55,56]. It can also be classified into four categories: 2D/3D structure similarity, computer-aided Quantitative Structure-Activity Relationship (QSAR), pharmacophore, and molecular docking [57,58]. Both advantages and disadvantages of the four approaches exist, and their prediction accuracy usually improves when combining two or more approaches.

Therefore, in the current study, three out of the four approaches were adopted, including the conformation-, QSAR- and pharmacophore-based approaches (Figure 1). Based on the three approaches, we built the “compound-targets-pathways” network. The analysis predicted that *Uncaria* alkaloids may affect “monoamine GPCRs”, “amine ligand-binding receptors”, “response to xenobiotic stimulus”, “blood circulation”, and “G protein-coupled serotonin receptor signaling pathway” to achieve the dual benefits in treating hypertension and alleviating AD at the same time (Figures 4 and 7). When the predicted results were further evaluated, BChE—one of the key targets related to the “response to xenobiotic stimulus” pathway—was picked for further validation. As a promising druggable target for AD, BChE activity is significantly elevated in later stages of AD [59–61]. Our study indicates that BChE may be a shared target for both AD and hypertension (Table 1). Hirsutine and hirsuteine—two *Uncaria* alkaloids from *Uncaria rhynchophylla*—showed a significant inhibitory effect on BChE activity (Figure 10). These results are consistent with the reported pharmacological effects of *Uncaria* extracts, and they demonstrated the efficiency and accuracy of our analytical platform on *Uncaria* alkaloids [6,9].

One of the limitations of this study is that the results from molecular docking cannot directly indicate the excitatory and inhibitory effects on the selected targets. Therefore, biological validation is usually performed to help evaluate and refine the results from the network pharmacology. Besides, the biological validation only covers a few selected *Uncaria* alkaloids with higher abundance. Therefore, the spectrum and potential of *Uncaria* alkaloids in treating AD and hypertension demonstrated here cannot be identical to the full effects of *Uncaria* plant extracts used clinically as a herbal medicine.

One more aspect to consider is the bioavailability of hirsutine and hirsuteine in vivo. One study from 2013 states that hirsutine and hirsuteine can be detected in rat plasma after oral administration of

a herbal formula containing *Uncaria* hooks [62]. Another study found the bioavailability of hirsutine and hirsutine via intravenous delivery is at 4.4% and 8.21%, respectively [63]. The pharmacokinetic (PK) properties of hirsutine, hirsuteine, and 3-dihydrocadambine (DHC) were also measured in our laboratory [64]. The AUC_{last} (hr*ng/mL) of the three alkaloids were 148.29 ± 54.11 , 1369.78 ± 736.15 , and 275.27 ± 52.19 , respectively [64]. Taking DHC as an example, despite its low bioavailability, our laboratory shows that DHC has a potent anti-hypertensive effect, which is consistent with a study by Aisaka et al. in 1985 [36]. Based on these studies, it is evident that there is a conflict between the low bioavailability of *Uncaria* alkaloids and their effectiveness in treating CNS symptoms (interpreted as “mood calming and mild sedation” based on *Uncaria*’s traditional application). It indicates that more analysis and in depth discussion are necessary to study the distribution and bioavailability of *Uncaria* alkaloids in the brain in our future studies.

In summary, our study has visualized an intricate network among *Uncaria* alkaloid compounds, their potential disease targets of AD and hypertension, and the possible pathways and biological processes. Though more biological validation is needed to further validate the current results, for the first time, the possibility of *Uncaria* alkaloids in delaying AD disease progression has been explored from the perspective of blood pressure control in a systemic approach. This combination of TCM and modern analytical methods may shine a new light on our approaches to studying TCM and provide a new therapeutic strategy and targets for patients who suffer from hypertension and AD.

4. Materials and Methods

4.1. Compound Library Construction and Drug-Likeness Screening

The in-house library of *Uncaria* alkaloids was compiled from previously published data from our laboratory (Table S1). *Uncaria* alkaloid compounds included in the library were first screened for their drug-likeness analysis using the FAFDrugs4 database [65]. The FAFDrugs4 database generates a computational prediction of drug likeness of the compounds to facilitate the drug-likeness screening. Duplicates and rejected molecules were discarded from the library, and only accepted compounds were included in the following analysis. Additional compounds were added back to the compound library because of their reported bioactivity from our earlier study [4]. Detailed information of the in-house library and accepted compounds is listed in the Supplementary Excel file.

4.2. Prediction of *Uncaria* Alkaloid Targets and Their Overall Biological Functions

All the information about the applied software, databases, and servers is listed in Table S2. Putative target prediction of the *Uncaria* alkaloids was made using an integrative application of TargetNet, SwissTargetPrediction, SEA, and PharmMapper servers (Table S2) [56,66–70]. These databases adopted various prediction algorithms and generated compound targets (genes). All the genes were presented in HGNC (HUGO Gene Nomenclature Committee) gene symbol format in the following analysis to avoid confusion across databases and platforms. These targets were pooled, and entries with “homo sapiens” origin were included in the following analysis. The overlaps were drawn using jvenn [71].

The above compound targets were further analyzed using the ClueGo plug-in of Cytoscape, and the overall protein–protein interaction (PPI) within the 85 *Uncaria* alkaloids and their targets were depicted (Table S2) [72,73].

4.3. Identification of Druggable Alzheimer’s Disease (AD) and Hypertension (HTN) Associated Disease Targets

To compile the disease targets for AD and hypertension, we searched with the keywords “Alzheimer’s disease” and “high blood pressure”/“hypertension” respectively in both DrugBank and TTD databases (Table S2) [74,75]. Only “approved” drugs with no limitations in “market availability” were included for the disease-related targets. For each disease, duplicated drug targets (genes) were removed. The overlapped and special targets in each target set were visualized using jvenn [71].

4.4. Network Construction, Topological Analysis and GO/KEGG Function Analysis

The “compound-disease targets” were the intersection of *Uncaria* alkaloid compound targets, AD disease targets, and HTN disease targets. In order to elucidate the interconnection between *Uncaria* alkaloid compounds, their potential target in AD and hypertension diseases, and the underlying biological mechanisms, a protein–protein interaction (PPI) network was constructed and analyzed with STRING and Cytoscape platforms (Table S2) [73,76]. The topological properties of every node in the interaction network were analyzed, and the results were ranked by “degree”, betweenness centrality (BC), degree centrality (DC), eigenvector centrality (EC), closeness centrality (CC), network centrality (NC), and local average connectivity (LAC). Nodes (targets) with higher ranks were considered to have a more critical role within the network.

To further find out the biological functions within the constructed network, GO/KEGG analyses for the compound-target network, target-disease network, and target-pathway network were performed with MetaScape, WebGestalt, and ConsensusPathDB respectively [77–79]. To analyze and visualize the GO/KEGG function, a GO Enrichment plot was created using ImageGP (<http://www.ehbio.com/ImageGP/>). The enriched target-pathway information was further visualized by WikiPathways database [80].

4.5. Molecular Docking

Molecular docking was performed with SYBYL-X Software (Version 2.0, Tripos International, St. Louis, MO, USA) using BCHE as a demonstration. 3D molecular structure was obtained from Pubchem website or drawn with ChemDraw (Version 19.0, PerkinElmer Informatics, Waltham, MA, USA), and then imported to SYBYL-X 2.0 for energy and conformation optimization with the following settings: “method: powell; termination: gradient; maximum iterations: 1000; force field: MMFF94; other parameters: default” [81]. Optimized small molecules were saved as mol₂ format. A Surflex-Dock approach was applied to analyze and repair the molecular structure of BCHE (RCSB PDB ID: 4TPK), and the docking targets were further defined [82]. The mol₂ file with information about the small molecules was applied to perform molecular docking on BCHE. The scores were collected for further analysis, and the results are summarized in Table 1.

4.6. In Vitro Butyrylcholinesterase (BChE) Enzyme Activity Assays

The enzymes BChE (Sigma-Aldrich, St. Louis, MO, USA) were dissolved in ultrapure water to make the 20 unit/mL stock solution, and the enzyme solutions were stored at –80 °C. The enzyme stock solution was diluted with 50 mM Tris-HCl (pH 7.8) before use. Solutions of tested compounds were prepared to start from 500 M stock solutions in dimethyl sulfoxide (DMSO; Thermo, Rockford, IL, USA) and diluted to a series of concentrations before the experiment. 5,5'-dithiobis-bis-nitrobenzoic acid (DTNB; Sigma-Aldrich, St. Louis, MO, USA) and butyrylthiocholine iodide (BTCI; Sigma-Aldrich, St. Louis, MO, USA) needed to be freshly dissolved in phosphate buffer (pH 7.6). The inhibitory activity of these *Uncaria* alkaloids against BChE was carried out in vitro using the Ellman colorimetric method with minor modifications [83]. Briefly, BTCI was used as substrate and DTNB as the chromophoric reagent. One hundred and forty µL phosphate buffer solution (pH 7.6), 20 µL 0.5 u/mL BChE, and 20 µL test compounds solution were added to the 96-well plate in turn. After being mixed and pre-incubated for 15 min at 25 °C, 10 µL 750 mM BTCI and 10 µL 500 mM DTNB were added to the mixture to start the reaction. After incubation for 30 min at 37 °C, the spectrophotometric absorption at 405 nm on an 800™ TS Absorbance Reader (Bio-Tek, Winooski, VT, USA) was measured immediately. 20 µL phosphate buffer solution (pH 7.6) was added to the blank group instead of the compounds to be tested. Galantamine (Selleck-China, Shanghai, China), a general cholinesterase inhibitor and FDA approved drug for AD treatment, was used as the positive control. The results of galantamine in the BChE activity assay was included in the Supplementary Materials (Table S3). All measurements were conducted in triplicate. Data were presented as mean ± S.D., and the IC₅₀ values were graphically

calculated from the inhibition curves by GraphPad Prism (Version 5.00, Graphpad Software, La Jolla, CA, USA).

Supplementary Materials: Supplementary materials can be found at <http://www.mdpi.com/1422-0067/21/5/1766/s1>.

Author Contributions: Conceptualization, W.W. (Wenyong Wu), Z.Z., and J.H.; methodology, W.W. (Wanying Wu) and Z.Z.; software, W.W. (Wanying Wu); validation, Z.Z., F.L., and Y.D.; formal analysis, W.W. (Wanying Wu) and Z.Z.; investigation, M.L.; resources, Y.D.; data curation, W.W. (Wanying Wu); writing—original draft preparation, Z.Z.; writing—review and editing, W.W. (Wanying Wu), Z.Z., and J.H.; visualization, X.X.; supervision, J.H.; project administration, H.L.; funding acquisition, W.W. (Wanying Wu). All authors have read and agree to the published version of the manuscript.

Funding: This research was funded by National Natural Science Foundation of China (Grant No. 81673591).

Acknowledgments: The authors are grateful for the financial support from the National Natural Science Foundation of China (Grant No. 81673591). We appreciate Huiqin Pan for her initial contribution in compiling the library of *Uncaria* alkaloids. We thank Lei Gao for her participation in the experiment discussion.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

AD	Alzheimer’s disease
BChE	butyrylcholinesterase
HTN	hypertension
PPI	protein–protein interaction
TCM	Traditional Chinese Medicine

References

- Zhang, Q.; Zhao, J.J.; Xu, J.; Feng, F.; Qu, W. Medicinal uses, phytochemistry and pharmacology of the genus *Uncaria*. *J. Ethnopharmacol.* **2015**, *173*, 48–80. [[CrossRef](#)] [[PubMed](#)]
- Laus, G. Advances in chemistry and bioactivity of the genus *Uncaria*. *Phytother. Res.* **2004**, *18*, 259–274. [[CrossRef](#)] [[PubMed](#)]
- Liu, L.; Zhao, Y.H.; Zeng, C.Q.; Zeng, Y. Research progress in pharmacological effects of *Uncaria Hook* on Alzheimer disease models. *Yao Xue Xue Bao* **2016**, *51*, 536–542. [[PubMed](#)]
- Feng, Z.; Hou, J.; Yu, Y.; Wu, W.; Deng, Y.; Wang, X.; Zhi, H.; Zhang, L.; Wu, W.; Guo, D.A. Dissecting the Metabolic Phenotype of the Antihypertensive Effects of Five *Uncaria* Species on Spontaneously Hypertensive Rats. *Front. Pharmacol.* **2019**, *10*, 845. [[CrossRef](#)] [[PubMed](#)]
- Ndagijimana, A.; Wang, X.; Pan, G.; Zhang, F.; Feng, H.; Olaleye, O. A review on indole alkaloids isolated from *Uncaria rhynchophylla* and their pharmacological studies. *Fitoterapia* **2013**, *86*, 35–47. [[CrossRef](#)]
- Fujiwara, H.; Iwasaki, K.; Furukawa, K.; Seki, T.; He, M.; Maruyama, M.; Tomita, N.; Kudo, Y.; Higuchi, M.; Saido, T.C.; et al. *Uncaria rhynchophylla*, a Chinese medicinal herb, has potent antiaggregation effects on Alzheimer’s beta-amyloid proteins. *J. Neurosci. Res.* **2006**, *84*, 427–433. [[CrossRef](#)]
- Yang, W.; Ip, S.P.; Liu, L.; Xian, Y.F.; Lin, Z.X. *Uncaria rhynchophylla* and Its Major Constituents on Central Nervous System: A Review on Their Pharmacological Actions. *Curr. Vasc. Pharmacol.* **2019**. [[CrossRef](#)]
- Shao, H.; Mi, Z.; Ji, W.G.; Zhang, C.H.; Zhang, T.; Ren, S.C.; Zhu, Z.R. Rhynchophylline Protects Against the Amyloid beta-Induced Increase of Spontaneous Discharges in the Hippocampal CA1 Region of Rats. *Neurochem. Res.* **2015**, *40*, 2365–2373. [[CrossRef](#)]
- Shin, S.J.; Jeong, Y.; Jeon, S.G.; Kim, S.; Lee, S.K.; Choi, H.S.; Im, C.S.; Kim, S.H.; Kim, S.H.; Park, J.H.; et al. *Uncaria rhynchophylla* ameliorates amyloid beta deposition and amyloid beta-mediated pathology in 5XFAD mice. *Neurochem. Int.* **2018**, *121*, 114–124. [[CrossRef](#)]
- Yang, Y.; Ji, W.G.; Zhu, Z.R.; Wu, Y.L.; Zhang, Z.Y.; Qu, S.C. Rhynchophylline suppresses soluble A β 1-42-induced impairment of spatial cognition function via inhibiting excessive activation of extrasynaptic NR2B-containing NMDA receptors. *Neuropharmacology* **2018**, *135*, 100–112. [[CrossRef](#)]
- Cao, J.; Hou, J.; Ping, J.; Cai, D. Advances in developing novel therapeutic strategies for Alzheimer’s disease. *Mol. Neurodegener.* **2018**, *13*, 64. [[CrossRef](#)] [[PubMed](#)]
- Pardridge, W.M. Alzheimer’s disease: Future drug development and the blood-brain barrier. *Expert Opin. Investig. Drugs* **2019**, *28*, 569–572. [[CrossRef](#)] [[PubMed](#)]

13. Sala Frigerio, C.; De Strooper, B. Alzheimer's Disease Mechanisms and Emerging Roads to Novel Therapeutics. *Annu. Rev. Neurosci.* **2016**, *39*, 57–79. [[CrossRef](#)] [[PubMed](#)]
14. Carnevale, D.; Perrotta, M.; Lembo, G.; Trimarco, B. Pathophysiological Links among Hypertension and Alzheimer's Disease. *High Blood Press. Cardiovasc. Prev.* **2016**, *23*, 3–7. [[CrossRef](#)]
15. Csiszar, A.; Tucsek, Z.; Toth, P.; Sosnowska, D.; Gautam, T.; Koller, A.; Deak, F.; Sonntag, W.E.; Ungvari, Z. Synergistic effects of hypertension and aging on cognitive function and hippocampal expression of genes involved in beta-amyloid generation and Alzheimer's disease. *Am. J. Physiol. Heart Circ. Physiol.* **2013**, *305*, H1120–H1130. [[CrossRef](#)]
16. Jeon, S.Y.; Byun, M.S.; Yi, D.; Lee, J.H.; Choe, Y.M.; Ko, K.; Sohn, B.K.; Choi, H.J.; Lee, J.Y.; Lee, D.Y.; et al. Influence of hypertension on brain amyloid deposition and Alzheimer's disease signature neurodegeneration. *Neurobiol. Aging* **2019**, *75*, 62–70. [[CrossRef](#)]
17. Sundar, U.; Manwatkar, A.A.; Joshi, A.R.; Bhandarkar, P. The Effect of Hypertension and Diabetes Mellitus on White Matter Changes in MRI Brain: A Comparative Study between Patients with Alzheimer's Disease and an Age-matched Control Group. *J. Assoc. Physicians India* **2019**, *67*, 14–17.
18. Shih, Y.H.; Wu, S.Y.; Yu, M.; Huang, S.H.; Lee, C.W.; Jiang, M.J.; Lin, P.Y.; Yang, T.T.; Kuo, Y.M. Hypertension Accelerates Alzheimer's Disease-Related Pathologies in Pigs and 3xTg Mice. *Front. Aging Neurosci.* **2018**, *10*, 73. [[CrossRef](#)]
19. Wiesmann, M.; Roelofs, M.; van der Lugt, R.; Heerschap, A.; Kiliaan, A.J.; Claassen, J.A. Angiotensin II, hypertension and angiotensin II receptor antagonism: Roles in the behavioural and brain pathology of a mouse model of Alzheimer's disease. *J. Cereb. Blood Flow Metab.* **2017**, *37*, 2396–2413. [[CrossRef](#)]
20. Kruyer, A.; Soplop, N.; Strickland, S.; Norris, E.H. Chronic Hypertension Leads to Neurodegeneration in the TgSwDI Mouse Model of Alzheimer's Disease. *Hypertension* **2015**, *66*, 175–182. [[CrossRef](#)]
21. Israeli-Korn, S.D.; Masarwa, M.; Schechtman, E.; Abuful, A.; Strugatsky, R.; Avni, S.; Farrer, L.A.; Friedland, R.P.; Inzelberg, R. Hypertension increases the probability of Alzheimer's disease and of mild cognitive impairment in an Arab community in northern Israel. *Neuroepidemiology* **2010**, *34*, 99–105. [[CrossRef](#)] [[PubMed](#)]
22. Yang, Y.H.; Roe, C.M.; Morris, J.C. Relationship between late-life hypertension, blood pressure, and Alzheimer's disease. *Am. J. Alzheimer's Dis. Other Dement.* **2011**, *26*, 457–462. [[CrossRef](#)] [[PubMed](#)]
23. Cifuentes, D.; Poittevin, M.; Dere, E.; Broqueres-You, D.; Bonnin, P.; Benessiano, J.; Pocard, M.; Mariani, J.; Kubis, N.; Merkulova-Rainon, T.; et al. Hypertension accelerates the progression of Alzheimer-like pathology in a mouse model of the disease. *Hypertension* **2015**, *65*, 218–224. [[CrossRef](#)] [[PubMed](#)]
24. Sato, T.; Hanyu, H.; Akai, T.; Takasaki, A.; Sakurai, H.; Iwamoto, T. A patient with early Alzheimer's disease who showed improvement of cognitive function and cerebral perfusion by combined therapy of nilvadipine and PPAR gamma agonists. *Nihon Ronen Igakkai Zasshi* **2008**, *45*, 428–433. [[CrossRef](#)]
25. Lawlor, B.; Kennelly, S.; O'Dwyer, S.; Cregg, F.; Walsh, C.; Coen, R.; Kenny, R.A.; Howard, R.; Murphy, C.; Adams, J.; et al. NILVAD protocol: A European multicentre double-blind placebo-controlled trial of nilvadipine in mild-to-moderate Alzheimer's disease. *BMJ Open* **2014**, *4*, e006364. [[CrossRef](#)] [[PubMed](#)]
26. Meulenbroek, O.; O'Dwyer, S.; de Jong, D.; van Spijker, G.; Kennelly, S.; Cregg, F.; Olde Rikkert, M.; Abdullah, L.; Wallin, A.; Walsh, C.; et al. European multicentre double-blind placebo-controlled trial of Nilvadipine in mild-to-moderate Alzheimer's disease-the substudy protocols: NILVAD frailty; NILVAD blood and genetic biomarkers; NILVAD cerebrospinal fluid biomarkers; NILVAD cerebral blood flow. *BMJ Open* **2016**, *6*, e011584. [[CrossRef](#)]
27. De Jong, D.L.K.; de Heus, R.A.A.; Rijppma, A.; Donders, R.; Olde Rikkert, M.G.M.; Gunther, M.; Lawlor, B.A.; van Osch, M.J.P.; Claassen, J. Effects of Nilvadipine on Cerebral Blood Flow in Patients with Alzheimer Disease. *Hypertension* **2019**, *74*, 413–420. [[CrossRef](#)]
28. Tu, Y. Artemisinin-A Gift from Traditional Chinese Medicine to the World (Nobel Lecture). *Angew. Chem. Int. Ed. Engl.* **2016**, *55*, 10210–10226. [[CrossRef](#)]
29. Kong, L.Y.; Tan, R.X. Artemisinin, a miracle of traditional Chinese medicine. *Nat. Prod. Rep.* **2015**, *32*, 1617–1621. [[CrossRef](#)]
30. Li, S. Advances in TCM symptomatology of rheumatoid arthritis. *J. Tradit. Chin. Med.* **2002**, *22*, 137–142.
31. Li, S.; Zhang, B. Traditional Chinese medicine network pharmacology: Theory, methodology and application. *Chin. J. Nat. Med.* **2013**, *11*, 110–120. [[CrossRef](#)] [[PubMed](#)]

32. Zhang, W.; Huai, Y.; Miao, Z.; Qian, A.; Wang, Y. Systems Pharmacology for Investigation of the Mechanisms of Action of Traditional Chinese Medicine in Drug Discovery. *Front. Pharmacol.* **2019**, *10*, 743. [[CrossRef](#)] [[PubMed](#)]
33. Lu, C.; Bing, Z.; Bi, Z.; Liu, M.; Lu, T.; Xun, Y.; Wei, Z.; Yang, K. Top-100 Most Cited Publications Concerning Network Pharmacology: A Bibliometric Analysis. *Evid. Based Complement. Alternat. Med.* **2019**, *2019*, 1704816. [[CrossRef](#)] [[PubMed](#)]
34. Zheng, J.; Wu, M.; Wang, H.; Li, S.; Wang, X.; Li, Y.; Wang, D.; Li, S. Network Pharmacology to Unveil the Biological Basis of Health-Strengthening Herbal Medicine in Cancer Treatment. *Cancers* **2018**, *10*, 461. [[CrossRef](#)]
35. Li, J.Y.; Chen, H.Y.; Dai, W.J.; Lv, Q.J.; Chen, C.Y. Artificial Intelligence Approach to Investigate the Longevity Drug. *J. Phys. Chem. Lett.* **2019**, 4947–4961. [[CrossRef](#)]
36. Aisaka, K.; Hattori, Y.; Kihara, T.; Ishihara, T.; Endo, K.; Hikino, H. Hypotensive action of 3alpha-dihydrocadambine, an indole alkaloid glycoside of *uncaria hooks*. *Planta Med.* **1985**, *51*, 424–427. [[CrossRef](#)]
37. Formagio, A.S.N.; Volobuff, C.R.F.; Kassuya, C.A.L.; Cardoso, C.A.L.; do Carmo Vieira, M.; Pereira, Z.V.; Bagatin, M.C.; de Freitas Gauze, G. Psychotria leiocarpa Extract and Vincosamide Reduce Chemically-Induced Inflammation in Mice and Inhibit the Acetylcholinesterase Activity. *Inflammation* **2019**, *42*, 1561–1574. [[CrossRef](#)]
38. Shi, J.S.; Huang, B.; Wu, Q.; Ren, R.X.; Xie, X.L. Effects of rhynchophylline on motor activity of mice and serotonin and dopamine in rat brain. *Zhongguo Yao Li Xue Bao* **1993**, *14*, 114–117.
39. Li, X.M.; Zhang, X.J.; Dong, M.X. Isorhynchophylline Attenuates MPP(+)-Induced Apoptosis through Endoplasmic Reticulum Stress- and Mitochondria-Dependent Pathways in PC12 Cells: Involvement of Antioxidant Activity. *Neuromol. Med.* **2017**, *19*, 480–492. [[CrossRef](#)]
40. Zhou, J.; Zhou, S. Antihypertensive and neuroprotective activities of rhynchophylline: The role of rhynchophylline in neurotransmission and ion channel activity. *J. Ethnopharmacol.* **2010**, *132*, 15–27. [[CrossRef](#)]
41. Shi, J.S.; Yu, J.X.; Chen, X.P.; Xu, R.X. Pharmacological actions of Uncaria alkaloids, rhynchophylline and isorhynchophylline. *Acta Pharmacol. Sin.* **2003**, *24*, 97–101. [[PubMed](#)]
42. Zhou, J.Y.; Zhou, S.W. Isorhynchophylline: A plant alkaloid with therapeutic potential for cardiovascular and central nervous system diseases. *Fitoterapia* **2012**, *83*, 617–626. [[CrossRef](#)] [[PubMed](#)]
43. Dams-O'Connor, K.; Guetta, G.; Hahn-Ketter, A.E.; Fedor, A. Traumatic brain injury as a risk factor for Alzheimer's disease: Current knowledge and future directions. *Neurodegener. Dis. Manag.* **2016**, *6*, 417–429. [[CrossRef](#)] [[PubMed](#)]
44. Caruso, A.; Nicoletti, F.; Mango, D.; Saidi, A.; Orlando, R.; Scaccianoce, S. Stress as risk factor for Alzheimer's disease. *Pharmacol. Res.* **2018**, *132*, 130–134. [[CrossRef](#)]
45. Norton, S.; Matthews, F.E.; Barnes, D.E.; Yaffe, K.; Brayne, C. Potential for primary prevention of Alzheimer's disease: An analysis of population-based data. *Lancet Neurol.* **2014**, *13*, 788–794. [[CrossRef](#)]
46. Fu, A.K.; Hung, K.W.; Huang, H.; Gu, S.; Shen, Y.; Cheng, E.Y.; Ip, F.C.; Huang, X.; Fu, W.Y.; Ip, N.Y. Blockade of EphA4 signaling ameliorates hippocampal synaptic dysfunctions in mouse models of Alzheimer's disease. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 9959–9964. [[CrossRef](#)]
47. Li, H.Q.; Ip, S.P.; Yuan, Q.J.; Zheng, G.Q.; Tsim, K.K.W.; Dong, T.T.X.; Lin, G.; Han, Y.; Liu, Y.; Xian, Y.F.; et al. Isorhynchophylline ameliorates cognitive impairment via modulating amyloid pathology, tau hyperphosphorylation and neuroinflammation: Studies in a transgenic mouse model of Alzheimer's disease. *Brain Behav. Immun.* **2019**, *82*, 264–278. [[CrossRef](#)]
48. Li, S.; Fan, T.P.; Jia, W.; Lu, A.; Zhang, W. Network pharmacology in traditional Chinese medicine. *Evid. Based Complement. Alternat. Med.* **2014**, *2014*, 138460. [[CrossRef](#)]
49. Hopkins, A.L. Network pharmacology. *Nat. Biotechnol.* **2007**, *25*, 1110–1111. [[CrossRef](#)]
50. Hopkins, A.L. Network pharmacology: The next paradigm in drug discovery. *Nat. Chem. Biol.* **2008**, *4*, 682–690. [[CrossRef](#)]
51. Chen, X.; Yan, C.C.; Zhang, X.; Zhang, X.; Dai, F.; Yin, J.; Zhang, Y. Drug-target interaction prediction: Databases, web servers and computational models. *Brief Bioinform.* **2016**, *17*, 696–712. [[CrossRef](#)] [[PubMed](#)]
52. Zhang, R.Z.; Yu, S.J.; Bai, H.; Ning, K. TCM-Mesh: The database and analytical system for network pharmacology analysis for TCM preparations. *Sci. Rep.* **2017**, *7*, 2821. [[CrossRef](#)] [[PubMed](#)]

53. Yu, G.; Wang, W.; Wang, X.; Xu, M.; Zhang, L.; Ding, L.; Guo, R.; Shi, Y. Network pharmacology-based strategy to investigate pharmacological mechanisms of Zuojinwan for treatment of gastritis. *BMC Complement. Altern. Med.* **2018**, *18*, 292. [[CrossRef](#)]
54. Lyu, M.; Zhou, Z.; Wang, X.; Lv, H.; Wang, M.; Pan, G.; Wang, Y.; Fan, G.; Gao, X.; Feng, Y.; et al. Network Pharmacology-Guided Development of a Novel Integrative Regimen to Prevent Acute Graft-vs.-Host Disease. *Front. Pharmacol.* **2018**, *9*, 1440. [[CrossRef](#)]
55. Keiser, M.J.; Setola, V.; Irwin, J.J.; Laggner, C.; Abbas, A.I.; Hufeisen, S.J.; Jensen, N.H.; Kuijjer, M.B.; Matos, R.C.; Tran, T.B.; et al. Predicting new molecular targets for known drugs. *Nature* **2009**, *462*, 175–181. [[CrossRef](#)]
56. Keiser, M.J.; Roth, B.L.; Armbruster, B.N.; Ernsberger, P.; Irwin, J.J.; Shoichet, B.K. Relating protein pharmacology by ligand chemistry. *Nat. Biotechnol.* **2007**, *25*, 197–206. [[CrossRef](#)] [[PubMed](#)]
57. Drwal, M.N.; Griffith, R. Combination of ligand- and structure-based methods in virtual screening. *Drug Discov. Today Technol.* **2013**, *10*, e395–e401. [[CrossRef](#)] [[PubMed](#)]
58. Sliwoski, G.; Kothiwale, S.; Meiler, J.; Lowe, E.W., Jr. Computational methods in drug discovery. *Pharmacol. Rev.* **2014**, *66*, 334–395. [[CrossRef](#)] [[PubMed](#)]
59. Sharma, P.; Srivastava, P.; Seth, A.; Tripathi, P.N.; Banerjee, A.G.; Shrivastava, S.K. Comprehensive review of mechanisms of pathogenesis involved in Alzheimer’s disease and potential therapeutic strategies. *Prog. Neurobiol.* **2019**, *174*, 53–89. [[CrossRef](#)]
60. Josviak, N.D.; Batistela, M.S.; Souza, R.K.M.; Wegner, N.R.; Bono, G.F.; Sulzbach, C.D.; Simao-Silva, D.P.; Piovezan, M.R.; Souza, R.L.R.; Furtado-Alle, L. Plasma butyrylcholinesterase activity: A possible biomarker for differential diagnosis between Alzheimer’s disease and dementia with Lewy bodies? *Int. J. Neurosci.* **2017**, *127*, 1082–1086. [[CrossRef](#)]
61. Macdonald, I.R.; Maxwell, S.P.; Reid, G.A.; Cash, M.K.; DeBay, D.R.; Darvesh, S. Quantification of Butyrylcholinesterase Activity as a Sensitive and Specific Biomarker of Alzheimer’s Disease. *J. Alzheimer’s Dis.* **2017**, *58*, 491–505. [[CrossRef](#)] [[PubMed](#)]
62. Kushida, H.; Fukutake, M.; Tabuchi, M.; Katsuhara, T.; Nishimura, H.; Ikarashi, Y.; Kanitani, M.; Kase, Y. Simultaneous quantitative analyses of indole and oxindole alkaloids of *Uncaria Hook* in rat plasma and brain after oral administration of the traditional Japanese medicine *Yokukansan* using high-performance liquid chromatography with tandem mass spectrometry. *Biomed. Chromatogr.* **2013**, *27*, 1647–1656. [[CrossRef](#)] [[PubMed](#)]
63. Han, A.; Lin, G.; Cai, J.; Wu, Q.; Geng, P.; Ma, J.; Wang, X.; Lin, C. Pharmacokinetic study on hirsutine and hirsuteine in rats using UPLC–MS/MS. *Acta Chromatogr.* **2019**, *31*, 99–104. [[CrossRef](#)]
64. Wang, J. *The Metabolic Study of Indole Alkaloids in Uncaria Rhynchophylla In Vivo*; China Pharmaceutical University: Nanjing, China, 2017.
65. Lagorce, D.; Bouslama, L.; Becot, J.; Miteva, M.A.; Villoutreix, B.O. FAF-Drugs4: Free ADME-tox filtering computations for chemical biology and early stages drug discovery. *Bioinformatics* **2017**, *33*, 3658–3660. [[CrossRef](#)] [[PubMed](#)]
66. Yao, Z.J.; Dong, J.; Che, Y.J.; Zhu, M.F.; Wen, M.; Wang, N.N.; Wang, S.; Lu, A.P.; Cao, D.S. TargetNet: A web service for predicting potential drug-target interaction profiling via multi-target SAR models. *J. Comput. Aided Mol. Des.* **2016**, *30*, 413–424. [[CrossRef](#)]
67. Daina, A.; Michielin, O.; Zoete, V. SwissTargetPrediction: Updated data and new features for efficient prediction of protein targets of small molecules. *Nucleic Acids Res.* **2019**, *47*, W357–W364. [[CrossRef](#)]
68. Gfeller, D.; Michielin, O.; Zoete, V. Shaping the interaction landscape of bioactive molecules. *Bioinformatics* **2013**, *29*, 3073–3079. [[CrossRef](#)]
69. Wang, X.; Shen, Y.; Wang, S.; Li, S.; Zhang, W.; Liu, X.; Lai, L.; Pei, J.; Li, H. PharmMapper 2017 update: A web server for potential drug target identification with a comprehensive target pharmacophore database. *Nucleic Acids Res.* **2017**, *45*, W356–W360. [[CrossRef](#)]
70. Liu, X.; Ouyang, S.; Yu, B.; Liu, Y.; Huang, K.; Gong, J.; Zheng, S.; Li, Z.; Li, H.; Jiang, H. PharmMapper server: A web server for potential drug target identification using pharmacophore mapping approach. *Nucleic Acids Res.* **2010**, *38*, W609–W614. [[CrossRef](#)]
71. Bardou, P.; Mariette, J.; Escudie, F.; Djemiel, C.; Klopp, C. jvenn: An interactive Venn diagram viewer. *BMC Bioinform.* **2014**, *15*, 293. [[CrossRef](#)] [[PubMed](#)]

72. Bindea, G.; Mlecnik, B.; Hackl, H.; Charoentong, P.; Tosolini, M.; Kirilovsky, A.; Fridman, W.H.; Pages, F.; Trajanoski, Z.; Galon, J. ClueGO: A Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. *Bioinformatics* **2009**, *25*, 1091–1093. [[CrossRef](#)] [[PubMed](#)]
73. Shannon, P.; Markiel, A.; Ozier, O.; Baliga, N.S.; Wang, J.T.; Ramage, D.; Amin, N.; Schwikowski, B.; Ideker, T. Cytoscape: A software environment for integrated models of biomolecular interaction networks. *Genome Res.* **2003**, *13*, 2498–2504. [[CrossRef](#)] [[PubMed](#)]
74. Wishart, D.S.; Knox, C.; Guo, A.C.; Cheng, D.; Shrivastava, S.; Tzur, D.; Gautam, B.; Hassanali, M. DrugBank: A knowledgebase for drugs, drug actions and drug targets. *Nucleic Acids Res.* **2008**, *36*, D901–D906. [[CrossRef](#)] [[PubMed](#)]
75. Chen, X.; Ji, Z.L.; Chen, Y.Z. TTD: Therapeutic Target Database. *Nucleic Acids Res.* **2002**, *30*, 412–415. [[CrossRef](#)] [[PubMed](#)]
76. Szklarczyk, D.; Gable, A.L.; Lyon, D.; Junge, A.; Wyder, S.; Huerta-Cepas, J.; Simonovic, M.; Doncheva, N.T.; Morris, J.H.; Bork, P.; et al. STRING v11: Protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res.* **2019**, *47*, D607–D613. [[CrossRef](#)]
77. Zhou, Y.; Zhou, B.; Pache, L.; Chang, M.; Khodabakhshi, A.H.; Tanaseichuk, O.; Benner, C.; Chanda, S.K. Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. *Nat. Commun.* **2019**, *10*, 1523. [[CrossRef](#)]
78. Liao, Y.; Wang, J.; Jaehnig, E.J.; Shi, Z.; Zhang, B. WebGestalt 2019: Gene set analysis toolkit with revamped UIs and APIs. *Nucleic Acids Res.* **2019**, *47*, W199–W205. [[CrossRef](#)]
79. Kamburov, A.; Stelzl, U.; Lehrach, H.; Herwig, R. The ConsensusPathDB interaction database: 2013 update. *Nucleic Acids Res.* **2013**, *41*, D793–D800. [[CrossRef](#)]
80. Slenter, D.N.; Kutmon, M.; Hanspers, K.; Riutta, A.; Windsor, J.; Nunes, N.; Melius, J.; Cirillo, E.; Coort, S.L.; Digles, D.; et al. WikiPathways: A multifaceted pathway database bridging metabolomics to other omics research. *Nucleic Acids Res.* **2018**, *46*, D661–D667. [[CrossRef](#)]
81. Kim, S.; Chen, J.; Cheng, T.; Gindulyte, A.; He, J.; He, S.; Li, Q.; Shoemaker, B.A.; Thiessen, P.A.; Yu, B.; et al. PubChem 2019 update: Improved access to chemical data. *Nucleic Acids Res.* **2019**, *47*, D1102–D1109. [[CrossRef](#)]
82. Berman, H.M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T.N.; Weissig, H.; Shindyalov, I.N.; Bourne, P.E. The Protein Data Bank. *Nucleic Acids Res.* **2000**, *28*, 235–242. [[CrossRef](#)] [[PubMed](#)]
83. Ellman, G.L.; Courtney, K.D.; Andres, V., Jr.; Feather-Stone, R.M. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.* **1961**, *7*, 88–95. [[CrossRef](#)]



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).