Network Pharmacology-Based Investigation on Therapeutic Mechanisms of the *Angelica dahurica* Radix and *Ligusticum chuanxiong* Rhizoma Herb Pair for Anti-Migraine Effect

Chu Duc Thanh 1,2, Chu Van Men 3, Hyung Min Kim 1,*, and Jong Seong Kang 1,*

1 College of Pharmacy, Chungnam National University, Daejeon 34114, Korea  
2 Institute of Biomedicine and Pharmacy, Vietnam Military Medical University, Hanoi 10000, Vietnam  
3 Clinical and Bioequivalence Testing Center, Vietnam Military Medical University, Hanoi 10000, Vietnam  
*Correspondence: kimhm@cnu.ac.kr (H.-M.K.); kangjss@cnu.ac.kr (J.-S.K.)

Abstract: Migraines are a common neurological disorder characterized by desperate throbbing unilateral headaches and are related to phonophobia, photophobia, nausea, and vomiting. The *Angelica dahurica* Radix and *Ligusticum chuanxiong* Rhizoma herb pair (ALHP) has been used to treat migraines for centuries in traditional Chinese medicine (TCM). However, the physiological mechanisms of migraine treatment have not yet been elucidated. In this study, a total of 50 hub targets related to the effect of 28 bioactive compounds in ALHP on anti-migraine were obtained through network pharmacology analysis. GO and KEGG analyses of the hub targets demonstrated that ALHP treatment of migraines significantly involved the G-protein-coupled receptor signaling pathway, chemical synaptic transmission, inflammatory response, and other biological processes. According to the degree of gene targets in the network, ACE, SLC3A6, NR3CI, MAPK1, PTGS2, PIK3CA, RELA, GRIN1, GRM5, IL1B, and DRD2 were found to be the core gene targets. The docking results showed a high affinity for docked conformations between compounds and predicted targets. The results of this study suggest that ALHP could treat migraines by regulating immunological functions, diminishing inflammation, and improving immunity through different physiological pathways, which contributes to the scientific base for more in-depth research as well as for a more widespread clinical application of ALHP.

Keywords: *Angelica dahurica*, *Ligusticum chuanxiong*, migraine, network pharmacology

1. Introduction

A migraine is a disabling primary headache that affects approximately 15% of the general population [1,2]. According to WHO reports [3], a migraine is ranked as the third most predominant medical disorder and the second neurological disease-induced disability worldwide [4,5]. The International Headache Society defines a migraine as an intensive throbbing headache that occurs with unilateral or bilateral localization [6]. Migraine Sphobia, nausea, and vomiting, as well as other neurological symptoms, such as tinnitus, dizziness, and cognitive impairment [7,8]. The principle treatment strategy for migraines aims to alleviate attack severity and duration, recover functioning ability, reduce the administration of medications, and expedite general management with minimal or no side effects [9,10]. In medication therapy for migraines, acute or abortive medications were usually prescribed for patients with infrequent migraine attacks, whereas the minority of preventive or prophylactic medications aimed to reduce the severity, duration or frequency of attacks in migraine patients [11]. Existing acute medications for migraines include non-steroidal anti-inflammatory drugs (NSAIDs), triptans (5-HT receptor agonists), calcitonin gene-related peptide (CGRP) receptor antagonists, and dopamine receptor antagonists [12]. In contrast, medications for migraine prophylaxis are categorized as beta-blockers, antidepressants, anticonvulsants, monoclonal antibodies against CGRP...
molecules, and receptors [12]. However, the unsatisfactory treatment efficacy of these medicines and their unpleasant adverse effects still requires prompt solutions [13]. Hence, there is an urgent demand for the discovery and development of novel and alternative migraine therapies to reduce the adverse effects of these medications.

Based on the TCM theory, migraines belong to the category of disease resulting from “Head Wind”. In this regard, wind phlegm, deficiency syndrome, and blood stasis syndrome are also considered the primary pathophysiological mechanisms of migraines [14]. Therefore, the critical viewpoints of TCM practice for migraine treatment aims to extinguish wind, resolve phlegm, activate blood, and relieve stasis. ALHP was passed down as an ancient Chinese prescription called “Duliang”. In the folk Chinese medicine literature, Bai Yi Xuan Fang compiled by Wang Miu (1196 A.D), the Duliang prescription for headache treatment was fully recorded and described in terms of both formulation and usage [15]. The prescription consisted of A. dahurica radix and L. chuanxiong rhizoma with a weight proportion of 4:1. It has also been approved by the China State Food and Drug Administration (statement number Z20000011) to treat symptoms such as stuffy nose, runny nose, and headaches since 2000 [15]. The rhizoma of A. dahurica Benth. et Hook. belongs to a perennial Apiaceae plant found abundantly in Taiwan, Korea, China, Japan, and Russia [16–18]. The antipyretic and analgesic properties of A. dahurica radix have been known for thousands of years [19]. This effect was proven to be based on the downregulation of the release of neurotransmitters and proinflammatory factors [20]. The rhizoma of L. chuanxiong Hort. is a commonly used traditional medicine for stimulating blood circulation and eliminating stasis in the clinical practice of TCM [21]. According to the approved Chinese Pharmacopoeia, L. chuanxiong rhizoma can be utilized to foster qi flow, blood circulation, wind expelling, and pain relief. It is often used to treat migraines, irregular menstruation, and rheumatism [22].

In TCM, the formula, which can be developed using one or several herbal components, contains many active ingredients. One ingredient might target one or multiple genes, proteins, and pathways in the pathogenic mechanism of a disease. Therefore, traditional herbal formulas can lead to an integrated or synergistic effect that is suitable for treating complex diseases. Network pharmacology is a recently developed method based mainly on the theory of systems biology and computer simulation technology [23]. The network pharmacology approach relies on the fundamental concept that multiple drugs in therapeutic fields act on multiple rather than single targets. By constructing the relationship between drugs, components, targets, and diseases, network pharmacology systematically investigates the multiple pharmacological effects of multiple components and multi-drug targets [24–26]. The investigation and analysis of the network of the interactions between multiple compounds, herbs, proteins, genes, and diseases, which is applied with network pharmacology approach, facilitates to elucidate the therapeutic efficacy of herbal formulas for disease [27,28].

In summary, this study aimed to exploit the network pharmacology approach to discover the potential bioactive compounds, core targets, and signaling pathways involved in the anti-migraine activity of ALHP. The results of this study may provide a theoretical basis for the molecular mechanism of ALHP in migraine treatment in future studies.

2. Results

2.1. Identification of the Main Active Compounds and Corresponding Targets

In accordance with the cut-off criteria of OB ≥30% and DL ≥0.18, 24, the main compounds of ALHP were obtained from the TCMSP database, including 19 compounds of A. dahurica radix and 6 compounds of L. chuanxiong rhizoma (one overlapping compound, mandenol). Four other compounds, daucosterol [29], ferulic acid [30], ligustilide [31,32], and senkynolide A [33], which did not meet the filtering criteria but have potential bioactivities related to migraine, were selected as potential compounds for further experiments. A list of these compounds is provided in Table 1. Based on the SwissTargetPrediction database, after removing targets with a probability of less than 0.1 and duplicated targets
between *A. dahurica* and *L. chuanxiong*, we obtained 655 targets corresponding to the compounds. The full names of the targets are displayed as gene symbols using the online UniProt database. The detailed data are shown in Table S1.

### 2.2. Identification of Target Genes Related to Migraines and the Overlapping Targets

Removing the targets with a relevance score lower than twice the median in the GeneCards database and then the duplicated targets between the GeneCards, OMIM, and DisGeNet databases induced a total of 979 targets that were considered candidate therapeutic targets (Table S2).

The targets of ALHP were intersected with those of migraines; 131 overlapping targets were determined (Table 2), and a Venn plot was drawn (Figure 1). These overlapping targets were considered potential targets in the therapeutic mechanism of ALHP against migraines.

**Figure 1.** Venn diagram of the overlapping target of ALHP and migraines.
### Table 1. The selected active compounds of ALHP.

| No. | Herb                | MOL ID     | Molecule Name                      | OB   | DL  | Smiles                                                                 |
|-----|---------------------|------------|------------------------------------|------|-----|-------------------------------------------------------------------------|
| 1   | *A. dahurica*       | MOL003791  | 2-linoleoylglycerol                | 37.28| 0.30| OCC(C)O(CCCCCC/C=C/C\C=C\CCCCC)=O                                     |
| 2   | *A. dahurica*       | MOL001939  | alloisoimperatorin                 | 34.80| 0.22| O=C1C=CC2=C(O)C3=C(O=C=C3)C/C=C(C\C=C\C)=C2O1                         |
| 3   | *A. dahurica*       | MOL000358  | beta-sitosterol                    | 36.91| 0.75| CCC(C=C(C)C1CCC2C1(CCCC3CCCC=C4C3(C=CC=C4)=O(=C(C3)(C=C(O)=C2O))C=C(C3)=O)|
| 4   | *A. dahurica*       | MOL005800  | byakangelicol                      | 41.42| 0.36| C1(C(O)1COC2=C3C(C4=C2O=C(O)=C4)OCC=C=C3=C(C)O                       |
| 5   | *A. dahurica*       | MOL000953  | cholesterol                        | 37.87| 0.68| CC(C)CCCCC(C)C1CCC2C1(CCCC3CCCC=C4C3(C=CC=C4)=O(=C(C3)(C=C(O)=C2O))C=C(C3)=O)|
| 6   | *A. dahurica*       | MOL001956  | cnidilin                           | 32.69| 0.28| O=C1C=CC2=C(OC/C=C(C)/C)C3=C(O=C=C3)C(OCC=C)=C2O1                     |
| 7   | *A. dahurica*       | MOL002883  | ethyl oleate                       | 32.40| 0.19| CCCCCC/C=C/C\CCCCCCCCC(OCC)=O                                          |
| 8   | *A. dahurica*       | MOL001941  | imperatorin                        | 34.55| 0.22| O=C1C=CC2=C3C=C(O=C=C3)(OC_OC=C(C)/C)=C2O1                             |
| 9   | *A. dahurica*       | MOL001942  | isoimperatorin                     | 45.46| 0.23| O=C(C=C1)OC2=C1OC(C=C(C)/C)=C(C=C3)=C3=C2                             |
| 10  | *A. dahurica*       | MOL007514  | methyl icosa-11,14-dienoate        | 39.67| 0.23| CCCCCC/C=C/C\CCCCCCCCC(OCC)=O                                          |
| 11  | *A. dahurica*       | MOL013430  | oxyimperatorin                     | 43.60| 0.29| O=C1C=CC2=C3C=C(OC=CC3=C(=OC)=C3)(OCC4C(C)=OC4)=C2O1                  |
| 12  | *A. dahurica*       | MOL002644  | phellopterin                       | 40.19| 0.28| O=C1OC2=C(OC/C=C(C)/C)C3=C(C=C3)=C(OCC=C)=C2C=C1                        |
| 13  | *A. dahurica*       | MOL003588  | prangenidin                        | 36.31| 0.22| O=C1C=CC2=C(C/C=C(C)/C)C3=C(OCC=C)=C2O1                               |
| 14  | *A. dahurica*       | MOL005802  | propyleneglycol monoolete         | 37.60| 0.26| CCCCCC=COC(C=OC)=OCC=C2=CC3=C(OC)=C2O1                                 |
| 15  | *A. dahurica*       | MOL005807  | sen-byakangelicol                 | 58.00| 0.61| O=C1C=CC2=C(OC3OC3C)=C4=C(OC=C)=C(OCC5OC5)=C2O1                        |
| 16  | *A. dahurica*       | MOL000449  | stigmasterol                       | 43.83| 0.76| CCC(C=CC(C)C1CCC2C1(CCCC3CCCC=C4C3(C=CC=C4)=O(=C(C3)(C=C(O)=C2O))C=C(C3)=O)|
| 17  | *A. dahurica*       | MOL001506  | supraene                           | 33.55| 0.42| C(C/C=C(C)/C)/C=C/C(C)/C=CC=C(C)/C/C=C(C)/C/C=C(C)\C=C(C)\C=C(C)\C=C(C)|
| 18  | *A. dahurica*       | MOL001749  | zinc3860434                        | 43.59| 0.35| CCCCCC(CCC)=OCC=C1=CC=CC=C1=CC=OOC(C=C)=C=C(CCC)=OOC(C[C@H=]CC)=OOC(C[C@H=]CC)=OOC(C=C)C(C)=O|
| 19  | *A. dahurica* / *L. chuanxiong* | MOL001494 | mandenol                           | 42.00| 0.19| CCCCCC/C=C/C\CCCCCCCCCCCC(OCC)=O                                          |
| 20  | *L. chuanxiong*     | MOL000433  | folic acid                         | 69.00| 0.71| C1=C(CC=C(CC)=O)NC(CCC(=O)=O)=O(NCC=C3=C=N2=C=N)=C(N3)=N               |
| 21  | *L. chuanxiong*     | MOL002135  | myricanone                          | 40.60| 0.51| COC1=CC(OC)=C2CCCCCC(CCC3=C(C1=C2)=C(O)=C3)=O                           |
| 22  | *L. chuanxiong*     | MOL002151  | senkyunone                         | 47.70| 0.24| O=C1C=C(C=C(C)=C(C)/C/C=CC(C)/C/C=CC(C)/C=CC(C)\C=C(C)\C=C(C)|
| 23  | *L. chuanxiong*     | MOL000359  | sitosterol                          | 36.90| 0.75| CCC(C=C(C)C1CCC2C1(CCCC3CCCC=C4C3(C=CC=C4)=O(=C(C3)(C=C(O)=C2O))C=C(C3)=O)|
| No. | Herb       | MOL ID     | Molecule Name | OB   | DL  | Smiles                                                                 |
|-----|------------|------------|---------------|------|-----|------------------------------------------------------------------------|
| 24  | *L. chuanxiong* | MOL002157  | wallichilide  | 42.3 | 0.71| CCCCC(=O)C12CCC(C=C1C(=O)OC)C3C2C4=C(C(C3)C(=CCCC)OC4=O               |
| 25  | *L. chuanxiong* | MOL000085  | daucosterol   | 20.63| 0.63| CCC(CCC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)OC5C(C(C(O5)CO)O)O)O)O)C(C(C)(C)C| |
| 26  | *L. chuanxiong* | MOL000360  | ferulic acid  | 55.14| 0.06| COC1=CC(/C=C/C(O)=O)=CC=C1O                                          |
| 27  | *L. chuanxiong* | MOL002201  | ligustilide   | 51.3 | 0.07| CCC=C1C2=C(C=C(C2)C(=O)O1                                            |
| 28  | *L. chuanxiong* | MOL002208  | senkynolide A | 26.6 | 0.07| CCCCC1C2=C(C=C(C2)C(=O)O1                                            |
Table 2. The overlapping targets between ALHP and migraines.

| Target  | Target  | Target  | Target  | Target  | Target  | Target  |
|---------|---------|---------|---------|---------|---------|---------|
| CA14    | PGR     | STAT6   | HTR2A   | ICAM1   | PDE2A   | SLC6A3  |
| AR      | PPARA   | TRPM8   | HTR6    | MMP9    | PDE5A   | CSNK1D  |
| SHBG    | PPARD   | TYMS    | HTR7    | SEL     | PIK3CB  | MAPK1   |
| RORA    | PPARG   | ACHE    | MAOB    | TRPV1   | TBXAS1  | P2RY12  |
| ESR1    | PTG52   | CHRM2   | MMP1    | UTS2R   | VCAM1   | PIK3CA  |
| ESR2    | ROUK2   | NO52    | MMP3    | MIP2    | BACE1   | SCN10A  |
| FAAH    | ADORA1  | SCN9A   | ATP      | CYP1A1  | SCNA2   | OPK1    |
| CYP19A1 | DRD3    | SLC6A4  | SLC2A1  | KNC1    | CYP1A2  | STK10   |
| CYP2C19 | EGFR    | VDR     | TAC1R   | TLR4    | F3      | TRPA1   |
| SLC6A2  | EphB2   | ADRA1B  | TGFBR2  | ADRA2B  | RELA    | ACE     |
| ADORA2A | JAK2    | DRD4    | ABCB1   | CCR5    | SLC16A1 | AGTR1   |
| CASR    | KCNMA1  | GABRA3  | ACACA   | CES1    | TTR     | KCNK9   |
| CNR1    | MAP2K2  | GABRA5  | DKK1    | EDNRA   | CCR1    | TRPV4   |
| F2R     | PIK3CD  | GABRG2  | DRD2    | ILIB    | F2      | STS     |
| HRH3    | PIK3CG  | GRM5    | HTR1A   | MGLL    | MAOA    | PTPRC   |
| NLRP3   | PRKCG   | HCRTR1  | HTR2C   | P2RX7   | PDGFRB  | INSR    |
| NR3C1   | RET     | HCRTR2  |         |         |         |         |

2.3. Construction of a Herb–Compound–Target–Disease Network

The efficacy of TCM prescriptions underlies the synergistic effect of various compounds in different herbs on multiple targets involved in a disease. Insight into the effects of compounds in ALHP on the target proteins of migraines may help clarify the mechanism of the synergistic effect and the potential mechanism of ALHP for migraine treatment. Therefore, the herb–compound–target–disease network associated with ALHP and migraines was analyzed using the Cytoscape software (Figure 2).

Figure 2. The herb–compound–target–disease network associated with ALHP and migraines.

2.4. Establishing PPI Network of Overlapping Targets and Selection of Hub Targets

The construction of the PPI network was implemented using the STRING web server. A total of 131 overlapping targets were entered into the STRING web server to yield a network with 131 nodes and 967 edges (Figure 3A). Thereafter, the PPI network was sent
to Cytoscape and analyzed via topological analysis to further illustrate the hub targets of ALHP and migraines. The nodes with BC, DC, and CC values lower than the median value were removed. As a result, 50 nodes and 402 edges were identified in the PPI network (network parameters: degree > 13, betweenness centrality > 0.005, closeness centrality > 0.44), as shown in Figure 3B and Table S3. Finally, cluster analysis of these identified targets set up four clusters (Cluster 1: EGFR, PGR, JAK2, PIK3CA, AGTR1, IL1B, MMP9, PPARA, MMP2, NOS2, REN, APP, PTPRC, AR, ACE, VCAM1, PTGS2, TLR4, F2, PPARG, and CXCL8; Cluster 2: TRPV1, NOS1, GRIN2A, HTR2C, HTR2A, SLC6A3, GRIA2; Cluster 3: RELA, MAPK1, ESR1, NR3C1, CYP19A1; and Cluster 4: CYP2C19, CNR1, ABCB1, ADORA2A, CYP1A2, OPRM1), which may express the interconnectivity and function of clustered proteins (Figure 3C). Especially, the nodes of gene targets ACE, SLC6A3, NR3C1, and ABCB1 were determined as the seed nodes with the highest scoring node in clusters 1, 2, 3, and 4, respectively (Table S3). The seed node, calculated and predicted via MCODE algorithm, might become the key target with high-probability in the cluster [34]. This suggests that these genes may be crucial to the therapeutic treatment of migraines with ALHP.

Figure 3. Identification of potential therapeutic targets for ALHP against migraines. (A) Construction of PPI networks of overlapping genes between ALHP and migraines via STRING. (B) Significant module determined via the function of topology analysis in Cytoscape. (C) Four clusters were predicted and visualized via the cluster analysis with MCODE algorithm (K-core threshold = 2). BC, betweenness centrality; CC, closeness centrality; DC, degree centrality.
2.5. GO Enrichment and KEGG Pathway Analysis

GO enrichment analysis was conducted using the DAVID web server to further elucidate the functions of the 50 hub genes. GO entries satisfying the criteria \(p < 0.01\), FDR < 0.05 included 218 biological processes, 46 cellular components, and 57 molecular functions (Table S4). The top 10 entries with the ordered \(-\log P\) value in each category (BP, CC, and MF) were selected, which showed that the hub genes were substantially enriched in MF, such as identical protein binding, protein serine/threonine/tyrosine kinase activity, enzyme binding, and neurotransmitter receptor activity, and in CC, such as plasma membrane, integral component of membrane, integral component of plasma membrane, cell surface, and BP, such as G-protein-coupled receptor signaling pathway, inflammatory response, cytokine-mediated signaling pathway, and chemical synaptic transmission (Figure 4).

Sixty KEGG pathways \((p < 0.01\), FDR < 0.05\) were obtained from the KEGG pathway analysis using DAVID. The elimination of obviously irrelevant KEGG pathways was performed, such as “pathways in cancer,” “Chagas disease,” and “hepatitis B”. The top 20 KEGG pathways with ranked \(p\) values were chosen and are shown in Figure 5 and Table S5. Accordingly, the potential KEGG pathways included neuroactive ligand–receptor interaction, pathways of neurodegeneration—multiple diseases, cAMP signaling pathway, calcium signaling pathway, estrogen signaling pathway, serotonergic synapse, Rap1 signaling pathway, TNF signaling pathway, inflammatory mediator regulation of TRP channels, NF-kappa B signaling pathway, Toll-like receptor signaling pathway, and HIF-1 signaling pathway. The top 20 KEGG pathways are listed in Table 3.
Figure 5. Top 20 KEGG pathways with ranked p value. The y-axis displays the KEGG pathway. The x-axis shows the amount of genes enriched in the pathway. The color represents the p-value, and the size of bubbles represents the amount of targets in the pathway. The color intensity from red to green shows the p value from high to low. The bigger the bubble size, the more targets in the pathway.

Table 3. Target genes in the top 20 of KEGG pathways.

| ID     | Term                                                                 | p-Value       | Genes                                                                 |
|--------|----------------------------------------------------------------------|---------------|----------------------------------------------------------------------|
| hsa04080 | Neuroactive ligand–receptor interaction                            | $1.14 \times 10^{-8}$ | GRIA2, HTR1A, HTR2C, TRPV1, HTR2A, OPRM1, F2, NR3C1, GRIN1, GRM5, GRIN2A, ADORA2A, CNR1, AGTR1, DRD2 |
| hsa05022 | Pathways of neurodegeneration—multiple diseases                     | $9.62 \times 10^{-5}$ | GRIA2, APP, GRM5, GRIN2A, NOS2, IL1B, MAPK1, NOS1, PTGS2, RELA, SLC6A3, GRIN1, VCAM1, CXCL8, PIK3CA, IL1B, CYP1A1, MAPK1, PPARG, JAK2, TLR4, MMP9, RELA |
| hsa05417 | Lipid and atherosclerosis                                           | $4.62 \times 10^{-7}$ | APP, GRM5, GRIN2A, PIK3CA, NOS2, IL1B, MAPK1, NOS1, PTGS2, RELA, GRIN1 |
| hsa05010 | Alzheimer disease                                                    | $7.87 \times 10^{-5}$ | APP, GRM5, GRIN2A, PIK3CA, NOS2, IL1B, MAPK1, NOS1, PTGS2, RELA, GRIN1 |
| hsa04024 | cAMP signaling pathway                                              | $5.59 \times 10^{-6}$ | GRIA2, GRIN2A, ADORA2A, PIK3CA, HTR1A, MAPK1, DRD2, PPARA, RELA, GRIN1 |
| hsa04020 | Calcium signaling pathway                                           | $1.09 \times 10^{-5}$ | GRM5, GRIN2A, ADORA2A, NOS2, AGTR1, HTR2C, NOS1, HTR2A, EGFR, GRIN1, PIK3CA, MMP2, MAPK1, PGR, OPRM1, ESR1, MMP9, EGFR, ESR2 |
| hsa04915 | Estrogen signaling pathway                                          | $1.43 \times 10^{-6}$ | APP, HTR1A, MAPK1, HTR2C, HTR2A, CYP2C19, PTGS2, SLC6A4 |
| hsa04726 | Serotonergic synapse                                                | $5.03 \times 10^{-6}$ | PIK3CA, NOS2, MMP2, MAPK1, NOS1, MMP9, RELA, EGFR |
| hsa04926 | Relaxin signaling pathway                                           | $1.08 \times 10^{-5}$ | PIK3CA, NOS2, MMP2, MAPK1, NOS1, MMP9, RELA, EGFR |
Table 3. Cont.

| ID     | Term                                             | p-Value   | Genes                                      |
|--------|--------------------------------------------------|-----------|--------------------------------------------|
| hsa04015 | Rap1 signaling pathway                           | $2.40 \times 10^{-4}$ | GRIN2A, ADORA2A, PIK3CA, CNR1, MAPK1, DRD2, EGFR, GRIN1 |
| hsa04668 | TNF signaling pathway                            | $5.22 \times 10^{-5}$ | VCAM1, PIK3CA, IL1B, MAPK1, PTGS2, MMP9, RELA |
| hsa04072 | Phospholipase D signaling pathway                | $2.44 \times 10^{-4}$ | GRM5, CXCL8, PIK3CA, AGTR1, MAPK1, F2, EGFR |
| hsa05030 | Cocaine addiction                               | $1.02 \times 10^{-5}$ | GRIA2, GRIN2A, DRD2, RELA, SLC6A3, GRIN1 |
| hsa04917 | Prolactin signaling pathway                     | $5.88 \times 10^{-5}$ | PIK3CA, MAPK1, JAK2, ESR1, RELA, ESR2 |
| hsa04540 | Gap junction                                     | $1.75 \times 10^{-4}$ | GRM5, MAPK1, HTR2C, HTR2A, DRD2, EGFR |
| hsa04657 | IL-17 signaling pathway                          | $2.39 \times 10^{-4}$ | CXCL8, IL1B, MAPK1, PTGS2, MMP9, RELA |
| hsa04750 | Inflammatory mediator regulation of TRP channels | $2.91 \times 10^{-4}$ | PIK3CA, TRPA1, IL1B, HTR2C, TRPV1, HTR2A |
| hsa04064 | NF-kappa B signaling pathway                     | $3.83 \times 10^{-4}$ | VCAM1, CXCL8, IL1B, PTGS2, TLR4, RELA |
| hsa04620 | Toll-like receptor signaling pathway             | $3.83 \times 10^{-4}$ | CXCL8, PIK3CA, IL1B, MAPK1, TLR4, RELA |
| hsa04066 | HIF-1 signaling pathway                          | $4.76 \times 10^{-4}$ | PIK3CA, NOS2, MAPK1, TLR4, RELA, EGFR |

2.6. Construction of Gene Target—Pathway Network

Gene target–pathway network analysis was constructed based on the enriched pathways and corresponding gene targets that regulated these pathways, as shown in Figure 6. The relationships between the top 20 KEGG pathways and their regulated gene targets are presented in the diagram. According to the results of the network analysis, MAPK1 has the largest size; hence, it was considered the core gene target. In addition, other gene targets were relatively large, including RELA, PIK3CA, EGFR, NOS2, and DRD2. These gene targets were counted as potential key gene targets involved in the ALHP treatment of migraines.

Figure 6. Gene target–pathway network of the ALHP against migraines. The light-yellow circles denote the target genes, and the pink squares denote pathways. Large size symbolizes larger degree.
2.7. Molecular Docking of the Bioactive Compounds of ALHP and Core Protein Targets

Furthermore, compounds with high network connectivity are important in disease treatment. In accordance with the number of connected targets, there were 13 active compounds with high connectivity in the herb–compound–target–disease network used for the molecular docking assay (Figure 7). In addition, fourteen potential targets, which not only were considered as the seed node in PPI and cluster analysis but also have a high degree in the KEGG pathway–target network, were selected for docking study, including ACE (PDB ID: 1O86), SLC6A3 (UniProt ID: Q01959), NR3C1 (PDB ID: 6YMO), ABCB1 (PDB ID: 6FN1), MAPK1 (PDB ID: 3SA0), PIK3CA (PDB ID: 3ZIM), RELA (PDB ID: 4KV1), IL1B (PDB ID: 5R8S), GRIN1 (PDB ID: 5KCJ), GRM5 (PDB ID: 6N4Y), PTGS2 (PDB ID: 5F19), DRD2 (PDB ID: 6CM4), HTR2C (PDB ID: 6BQG), and NO52 (PDB ID: 3HR4). Among these target proteins, only the target SLC6A3 was modeled using the SWISS-MODEL and successfully validated via the Verify3D server (GMQE = 0.72; 87% of the residues with 3D-1D score ≥ 0.2). According to the binding energy in the docking assay, binding with a lower energy value is consistent with a stronger binding force to the protein. Generally, a binding capacity lower than −5.0 kcal/mol implies strong docking of conformation between ligand and protein, and lower values indicate stronger binding. The root mean square deviation (RMSD) values of the docking model for each compound were less than 2 Å, which confirmed that all the docking models were reliable [35]. Among the bioactive compounds, sen-byakangelicol, imperatorin, oxyimperatorin, cnidilin, ferulic acid, ligustilide, phellopterin, senkyunolide A, senkyunone, and wallichilide showed a high binding capacity for all 14 protein targets, whereas other compounds, such as zinc3860434, 2-linoleoylglycerol and propyleneaglycerol monolate, showed good interactions with only several targets (Figure 7). The detailed docking results of all 13 bioactive compounds with protein targets that showed the highest binding were visualized using PyMOL and Discovery Studio Visualizer software, as shown in Figure 8.

Figure 7. Heatmap of binding score (kcal.mol\(^{-1}\)) between compound and protein target in the molecular docking study. The RMSD values below 2.0 Å indicate the docking results are acceptable.
Figure 8. Two-dimensional visualization data of the compound–protein complex in the docking study.
3. Discussion

Migraines are one of the major causes of human disability worldwide [5]. To date, the clinical effectiveness of available remedies for migraine patients has been restricted due to poor efficacy, inescapable adverse effects, and medication abuse [13]. Thus, modern medicine still faces a huge challenge in the prevention and treatment of migraines. According to TCM principles, ALHP is effective in modulating qi flow, enhancing blood circulation, and soothing headaches. It has been commonly administered to alleviate different types of pain caused by qi and blood stasis conditions. Although ALHP manipulated in different dosage forms, such as pills, coated pills, and soft capsules, is broadly prescribed by TCM physicians, the pharmacodynamic material cause and mechanisms of action need more in-depth study.

Network pharmacology is a growing field that is widely applied in the field of drug discovery. This study employed integrated network pharmacology and molecular docking approaches to explore the molecular mechanisms of ALHP in migraine treatment. The findings showed that ALHP exerts a potential role in treating migraines by regulating multiple target genes, including ACE, SLC6A3, NR3C1, HTR2A, HTR2C, GRIN1, GRIN2A, DRD2, MAPK1, IL1B, RELA, NOS2, and PIK3CA.

The traditional use of *A. dahurica* radix as a remedy for headache and migraine has been documented in the folk literature and recent studies. The main chemical composition of *A. dahurica* includes coumarins with anti-oxidant and anti-inflammatory activities, namely imperatorin and oxyimperatorin, which are predicted to play key roles in migraine treatment [36,37]. In addition, phellopterin and cnidilin were detected as the major compounds in the TCM formula extract with anti-migraine activity [38]. The active ingredients in *L. chuanxiong* rhizoma, such as ferulic acid, senkyunone, ligustilide, and senkyunolide A, exhibited anti-inflammatory and anti-migraine activities and effectively prevented ischemic events [39,40].

Regarding the key targets, the angiotensin-converting enzyme (ACE) serves a primary role in stimulating inactive angiotensin I to active angiotensin II, a vasoconstrictor. Vasoconstrictor were early proved to cease migraine attacks [41,42]. The mitogen-activated protein kinase 1 (MAPK1), a member of the MAPK family, and MAP kinases are involved in many cellular signaling processes, such as proliferation and transcription regulation. Activated MAPK is proposed to modulate the synthesis and release of the neuropeptide calcitonin gene-related peptide (CGRP), which is associated with the pathogenesis of migraines [43,44]. In trigeminal ganglia neurons, MAPKs stimulate CGRP transcription via enhancer control [45]. Thus, the results of this study prove that MAPK1 targets mediating migraines via the MAPK signaling pathway, which is consistent with published studies. The increased level of peripheral proinflammatory cytokines involving IL1B enables an increase in the neuronal conduction of peripheral nociceptive neurons and, thus, a more significant peripheral nociceptive input, which may be attributed to central sensitization and improved hyperalgesia in the literature on chronic tension-type headaches [46]. In recently published studies, excessive serum levels of IL1B (proinflammatory cytokine) in patients suffering from migraines revealed that migraines had a tightened association with inflammation occurring within the peripheral endings of sensory neurons in the trigeminal ganglion system [47]. NR3C1 (glucocorticoid receptor) has effects on inflammatory responses, and especially has a wide distribution in neurons and neuroglia, which shows the active role of NR3C1 in migraines [48,49]. RELA was identified as a monomer in combination with other members of the Rel-like domain-containing proteins, such as RELB, NFKB1/p105, NFKB1/p50, REL, and NFKB2/p52, in order to form a homo- or heterodimeric complex of nuclear factor kappa B (NF-kappa-B). NF-kappa-B, a transcription factor involved in the inflammatory response, has been suggested as a mediator of the neurochemical cascade causing migraine attacks [50,51]. NOS enzymes, including NOS2, inhibit nitric oxide biosynthesis, thereby possibly functioning at peripheral locations to inhibit neurogenic dural vasodilation and at the endothelial level to hinder the dilation induced by CGRP [52]. PIK3CA functions as a catalytic subunit of phosphatidylinositol
3-kinase (PI3K), which phosphorylates signaling molecules through the PI3K pathway. In a rat model of migraines, activation of the PI3K/AKT signaling pathway may be triggered in the brain tissue [33]. Much preclinical and clinical evidence suggests that neurotransmitters and receptors, such as serotonin (5-HT), dopamine, and glutamate, are involved in migraine pathophysiology [54,55]. Consequently, the target SLC6A3 (dopamine transporter) fully participates in the pathogenesis of migraines. GRIN1, GRIN2A, and GRM5 are ionotropic and metatropic glutamate receptors, respectively. The development of glutamate receptor antagonists is one of the therapies for migraine treatment [56]. In addition, dopamine receptors play a significant role in migraine pathogenesis. A large number of studies have focused on the function of dopamine receptor D2 (DRD2) in central nervous system disorders, such as movement disorders, schizophrenia, migraine, and posttraumatic stress disorder [57,58]. Briefly, 5-HT and its receptors, such as HTR2A, HTR2C are implicated in migraines [54].

In terms of the pathway to further determine the therapeutic mechanisms of the ALHP formula, our study focused on the canonical KEGG pathways possibly linked to anti-migraine treatment and prophylaxis. In the serotonergic synapse pathway, the intracellular network cascade is triggered by serotonin, resulting in repressive or excitatory neurotransmission. The dispersion of serotonin receptors occurs in the brain, pain-signaling circuits, and cranial blood vessels. Anti-migraine therapies have been used to modulate serotonin receptors [55]. These pathways can be related to glial cell activation (neuroactive ligand-receptor interaction, cAMP signaling pathway, calcium signaling pathway, and gap junction), neuroinflammation (estrogen signaling pathway, NF-kappa B signaling pathway, TNF signaling pathway, Toll-like receptor signaling pathway, and Alzheimer’s disease pathway) [59], and neuro-immune responses (prolactin signaling pathway and cocaine addiction pathway) [60,61]. The Neuroactive ligand–receptor interaction signaling pathway is directly related to neurofunctions [62]. Neuroactive ligands binding to intracellular receptors affect neuronal function, which results from either binding transcription factors or regulating gene expression [63]. Neuroactive steroids act as hormones that regulate neurotransmitter receptors to either stimulate or inhibit neuronal activity [64]. It has been shown that the cAMP and possibly cGMP signaling pathway are associated with the activation of KATP channels. KATP channels are thought to be related to the pathophysiology of migraines through their function in the cerebral and meningeal arteries as well as the trigeminal system [65].

This study hypothesized that the anti-migraine effect of ALHP may be exerted mainly via the regulation of neuroactive ligand–receptor interaction, pathways of neurodegeneration—multiple diseases, serotonergic synapses, cAMP, and calcium signaling pathways. In addition, as holistic medicine, the anti-migraine mechanism of ALHP possibly acts through the NF-kappa B, TNF, cAMP, HIF-1, Toll-like receptor, and calcium signaling pathways to moderate the neurovascular systems and through neuro-inflammation and pain-related proteins, which produces a synergistic effect to relieve the burden of migraines.

4. Materials and Methods

The workflow of network pharmacology approach included these steps: (1) Compounds of ALHP were collected using the database of medicinal herbs and text mining. (2) Information about gene targets related to migraine disease was also retrieved in free and updated databases of human gene and diseases. (3) The overlapping targets were determined using a Venn diagram. (4) Topology analysis including the PPI analysis and network construction was carried out. (5) Core targets and compounds were screened and determined with threshold criteria of degree, closeness, and betweenness centrality. (6) GO and KEGG pathway analysis and molecular docking assay were performed on the potential compounds and core gene targets. The process of network pharmacology is described and summarized in Figure 9.
We collected targets related to migraines from three data sources: GeneCards (https://www.genecards.org (accessed on 20 November 2021)), DisGeNET (https://www.disgenet.org/home/ (accessed on 20 November 2021)), and OMIM (https://omim.org (accessed on 23 November 2021)). The keyword “migraine” was entered and searched for in each database. The GeneCards database, which was automatically mined and integrated from 150 web sources, provides user-friendly and comprehensive information regarding disease targets annotated and predicted in the human species. The wealth of GeneCards annotation was exploited with the GeneCards Inferred Functionality Score (GIFTs) algorithm to yield scores to predict the degree of functionality of the target. Based on the general criteria of GeneCards Inferred Functionality Score (GIFTs), the target with a score ≥30 was identified as the criteria target [76,77]. The Online Mendelian Inheritance in Man (OMIM) database, which is freely available and updated daily, contains information regarding known diseases and the corresponding genes in the genome of our species and the relationship between
phenotype and genotype [78,79]. DisGeNET, a platform with comprehensive multifunctional data, integrates and processes information on human disorders and target genes to reveal the relationships between diseases and targets [80]. Combining targets obtained from the three databases and removing duplicates induced a set of potential targets associated with migraines.

4.3. Construction of Herb–Compound–Target–Disease Network and PPI Network

The overlapping targets from the two sets of targets of compounds and diseases were determined using the Venny tool (http://bioinfogp.cnb.csic.es/tools/venny/index.html (accessed on 25 November 2021)). The herb–compound–target–disease network was established and visually displayed using Cytoscape software (Cytoscape, Seattle, WA, USA, version 3.9.1, https://cytoscape.org/ (accessed on 26 August 2021)) with information input formats such as source node, target node, and source node attribute.

The overlapping targets were imported into the STRING database (https://string-db.org/ (accessed on 14 December 2021)), and a protein–protein interaction (PPI) network was constructed with the following screening conditions: the species as “Homo sapiens”, the required interaction score at the level of medium confidence (0.400), and other parameters in default mode [81]. In the PPI plot, each node represents a gene, and the nodes are connected by edges. For further study of the PPI network, the PPI results in STRING were transferred to the Cytoscape software. The function “Analyze Network” in Cytoscape calculates the topological properties of a node in a network, namely degree centrality (DC), betweenness centrality (BC), and closeness centrality (CC). In addition, the app “ClusterViz” with the MCODE algorithm in Cytoscape was also used to clarify highly interconnected regions, or clusters, of the network, as well as to calculate and predict the seed node of cluster [34,82]. The default parameters optimized in the MCODE algorithm includes: Include Loop = false (off or unselected); Degree Threshold = 2; Haircut = true (on or selected); Fluff = false (off or unselected); NodeScore Threshold = 0.2; K-Core Threshold = 2; and MaxDepth = 100 [83,84].

4.4. Functional Enrichment Analysis of GO and KEGG Pathway

Gene Ontology (GO) functions and KEGG signaling pathways with potential targets were enriched using the Database for Annotation, Visualization, and Integrated Discovery (DAVID, https://david.ncifcrf.gov/ (accessed on 19 January 2022)) [85]. DAVID, an online bioinformatics resource, aims to interpret the functions of the submitted set of genes. In the DAVID analysis, the species as “Homo sapiens” was selected as the screening criterion. In addition, the dissimilarity in GO terms and KEGG signaling pathways with a false discovery rate (FDR) value of < 0.05 was considered significant. Finally, the bubble diagram of KEGG pathways was plotted using the ggplot2 package in the R language.

4.5. Molecular Docking of the Main Bioactive Compounds of ALHP and Core Target Proteins

A molecular docking study was performed to validate the association of compounds with key targets in the pathogenesis pathways in a network pharmacology study. The Avogadro program was utilized to form the 3D chemical structures of molecular ligands via the input of molecules in the SMILES format and auto-optimization function [86]. The three-dimensional (3D) structure of the protein receptor was obtained from the PDB online database (http://www.rcsb.org/ (accessed on 3 February 2022)). In another way, the 3D model of protein, based on the amino acid sequence from UniProt database [87] (https://www.uniprot.org/ (accessed on 2 December 2021)), was also built via the online server SWISS-MODEL (https://swissmodel.expasy.org/ (accessed on 3 February 2022)) and validated using the Verify3D Structure Evaluation Server (https://www.doe-mbi.ucla.edu/verify3d/ (accessed on 3 February 2022)) [87–89]. To remove molecular ligands and water from the protein receptor, the PyMol 2.4.0 program (https://pymol.org (accessed on 9 February 2022)) was utilized. The format of the receptor and ligand was transformed into pdbqt format via AutoDockTools 1.5.6 software. Active-binding pockets were identified.
Subsequently, molecular docking was performed and calculated using Perl scripts in AutoDock Vina [90]. Finally, docking affinity was determined by selecting the affinity with the lowest binding energy, and the root mean square deviation (RMSD) values of all docked poses were measured by the RMSD/Superimpose function in AutoDock Tools. In data visualization, the 3D conformation structures of the ligands and receptors were displayed using PyMol software [91]. Discovery Studio Visualizer v21.1 software enabled the interaction between the protein and ligand to be visualized as a 2D image [92].

5. Conclusions

Using computational methods, including network pharmacology combined with molecular docking, this study revealed that the ALHP formula exerts an anti-migraine effect by regulating multiple targets and pathways in the pathogenesis of migraines. Among the components of the ALHP formula, imperatorin, ligustilide, oxyimperatorin, phellopterin, sen-byakangelicol, cnidilin, ferulic acid, senkyunolide A, senkyunone, and wallichilide were expressed in various associations in the pathophysiological pathways of migraines, which are considered as biomarkers of the formula. In addition, our study will provide a scientific basis for more comprehensive research and for a more widespread clinical application of ALHP in migraine treatment.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/plants11172196/s1; Table S1. Compound Targets of A. dahurica, L. chuanxiong and ALHP; Table S2. Targets of Migraine; Table S3. Hub targets and Clusters in PPI analysis; Table S4. GO analysis; Table S5. KEGG analysis.

Author Contributions: Conceptualization, C.D.T., C.V.M., H.-M.K., and J.-S.K.; data curation, C.D.T.; formal analysis, C.D.T.; methodology, C.D.T. and C.V.M.; project administration, H.-M.K. and J.-S.K.; supervision, H.-M.K. and J.-S.K.; funding acquisition, J.-S.K. and H.-M.K.; writing—original draft, C.D.T.; writing—review and editing, C.D.T., C.V.M., H.-M.K., and J.-S.K. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the BK21 FOUR Program by the Chungnam National University Research Grant, 2022 and by the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Education (NRF-2021R1H1A3047248).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article and Supplementary Material.

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

References

1. Ashina, M.; Hansen, J.M.; Do, T.P.; Melo-Carrillo, A.; Burstein, R.; Moskowitz, M.A. Migraine and the Trigeminovascular System—40 Years and Counting. Lancet Neurol. 2019, 18, 795–804. [CrossRef]
2. Feigin, V.L.; Nichols, E.; Alam, T.; Bannick, M.S.; Beghi, E.; Blake, N.; Culpepper, W.J.; Dorsey, E.R.; Elbaz, A.; Ellenbogen, R.G.; et al. Global, Regional, and National Burden of Neurological Disorders, 1990–2016: A Systematic Analysis for the Global Burden of Disease Study 2016. Lancet Neurol. 2019, 18, 459–480. [CrossRef]
3. World Health Organization. General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine; World Health Organization: Geneva, Switzerland, 2000.
4. Feigin, V.L.; Krishnamurthi, R.V.; Theadom, A.M.; Abajobir, A.A.; Mishra, S.R.; Ahmed, M.B.; Abate, K.H.; Mengistie, M.A.; Wakayo, T.; Abd-Allah, F.; et al. Global, Regional, and National Burden of Neurological Disorders during 1990–2015: A Systematic Analysis for the Global Burden of Disease Study 2015. Lancet Neurol. 2019, 18, 459–480. [CrossRef]
5. James, S.L.; Abate, D.; Abate, K.H.; Ayab, S.M.; Abbafati, C.; Abbasi, N.; Abbastabar, H.; Abd-Allah, F.; Abdela, J.; Abdalalim, A.; et al. Global, Regional, and National Incidence, Prevalence, and Years Lived with Disability for 354 Diseases and Injuries for 195 Countries and Territories, 1990–2017: A Systematic Analysis for the Global Burden of Disease Study 2017. Lancet 2018, 392, 1789–1858. [CrossRef]
6. Olesen, J. Headache Classification Committee of the International Headache Society (IHS) The International Classification of Headache Disorders, 3rd Edition. Cephalalgia 2018, 38, 1–211. [CrossRef]
7. Dodick, D.W. Migraine. Lancet 2018, 391, 1315–1330. [CrossRef]
8. Dodick, D.W.; Lipton, R.B.; Ailani, J.; Lu, K.; Finnegan, M.; Tragman, J.M.; Szegedi, A. Ubrogepant for the Treatment of Migraine. *N. Engl. J. Med.* 2019, 381, 2230–2241. [CrossRef] [PubMed]

9. Marmura, M.J.; Silberstein, S.D.; Schwedt, T.J. The Acute Treatment of Migraine in Adults: The American Headache Society Evidence Assessment of Migraine Pharmacotherapies. *Headache* 2015, 55, 3–20. [CrossRef]

10. Silberstein, S.D.; Edlund, W. Practice Parameter: Evidence-Based Guidelines for Migraine Headache (an Evidence-Based Review): Report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology* 2000, 55, 754–762. [CrossRef]

11. Peters, G.L. Migraine Overview and Summary of Current and Emerging Treatment Options. *Am. J. Manag. Care* 2019, 25, S23–S34.

12. Singh, A.; Gupta, D.; Sahoo, A.K. Acute Migraine: Can the New Drugs Clinically Outpace? *SN Compr. Clin. Med.* 2020, 2, 1132–1138. [CrossRef]

13. May, A.; Schulte, J.H. Chronic Migraine: Risk Factors, Mechanisms and Treatment. *Nat. Rev. Neurol.* 2016, 12, 455–464. [CrossRef]

14. Huang, Y.; Ni, N.; Hong, Y.; Lin, X.; Feng, Y.; Shen, L. Progress in Traditional Chinese Medicine for the Treatment of Migraine. *Am. J. Chin. Med.* 2020, 48, 1731–1748. [CrossRef]

15. Yu, S.; Hu, Y.; Wan, Q.; Zhou, J.; Liu, X.; Qiao, X.; Yang, X.; Feng, J.; Chen, K.; Pan, X.; et al. A Multicenter, Double-Blind, Randomized, Placebo-Controlled Trial to Evaluate the Efficacy and Safety of Duliang Soft Capsule in Patients with Chronic Daily Headache. *Evid. Based Complement. Altern. Med.* 2015, 2015, 694061. [CrossRef]

16. Wang, N.H.; Huang, L.Q.; Yang, B.; Baba, K.; Taniguchi, M.; Yuan, C.Q.; Qin, H.Z.; Shu, P. Studies on Original Plant of Traditional Chinese Drug “Bai Zhi” (Radix Angelicae Dahuricae) and Its Closely Related Wild Plants. *China J. Chin. Mater. Med.* 2001, 26, 733–736.

17. Sarker, S.; Nahar, L. Natural Medicine: The Genus Angelica. *Curr. Med. Chem.* 2004, 11, 1479–1500. [CrossRef]

18. Li, X.; Zeng, X.; Sun, J.; Li, H.; Wu, P.; Fung, K.P.; Liu, F. Imperatorin Induces McI-1 Degradation to Cooperatively Trigger Bax Translocation and Bak Activation to Suppress Drug-Resistant Human Hepatoma. *Cancer Lett.* 2014, 348, 146–155. [CrossRef]

19. Le, J.; Lin, Z.; Song, L.; Wang, H.; Hong, Z. LC-MS/MS Combined with in Vivo Microdialysis Sampling from Conscious Rat Striatum for Simultaneous Determination of Active Constituents of Yuanhu-Baizhi Herb Pair and Endogenous Neurotransmitters: Application to Pharmacokinetic and Pharmacodynamic Study. *J. Pharm. Biomed. Anal.* 2019, 176, 112807. [CrossRef]

20. Wang, N.H.; Huang, L.Q.; Yang, B.; Baba, K.; Taniguchi, M.; Yuan, C.Q.; Qin, H.Z.; Shu, P. Studies on Original Plant of Traditional Chinese Drug “Bai Zhi” (Radix Angelicae Dahuricae) and Its Closely Related Wild Plants. *China J. Chin. Mater. Med.* 2001, 26, 733–736.

21. Shan Feng, J.H. Herbal Textual Research on Origin and Development of Chuanxiong. *China J. Chin. Mater. Med.* 2011, 36, 2306–2310.

22. Chinese Pharmacopoeia Commission. *Pharmacopoeia of the People’s Republic of China 2020—Part I*; Chinese Medical Science and Technology Press: Beijing, China, 2020.

23. Hopkins, A.L. Network Pharmacology. *Nat. Biotechnol.* 2007, 25, 1110–1111. [CrossRef]

24. Li, S.; Zhang, B. Traditional Chinese Medicine Network Pharmacology: Theory, Methodology and Application. *Chin. J. Nat. Med.* 2013, 11, 110–120. [CrossRef]

25. Zhang, G.-B.; Li, Q.-Y.; Chen, Q.-L.; Su, S.-B. Network Pharmacology: A New Approach for Chinese Herbal Medicine Research. *Evid.-Based Complement. Altern. Med.* 2013, 2013, 621423. [CrossRef]

26. Muhammad, J.; Khan, A.; Ali, A.; Fang, L.; Yanjing, W.; Xu, Q.; Wei, D.-Q. Network Pharmacology: Exploring the Resources and Methodologies. *Curr. Top. Med. Chem.* 2018, 18, 949–964. [CrossRef]

27. Wu, X.-M.; Wu, C.-F. Network Pharmacology: A New Approach to Unveiling Traditional Chinese Medicine. *Chin. J. Nat. Med.* 2015, 13, 1–2. [CrossRef]

28. Zhang, R.; Zhu, X.; Bai, H.; Ning, K. Network Pharmacology Databases for Traditional Chinese Medicine: Review and Assessment. *Front. Pharmacol.* 2019, 10, 123. [CrossRef]

29. Li, Y.; Zhang, J.; Zhang, L.; Chen, X.; Pan, Y.; Chen, S.S.; Zhang, S.; Wang, Z.; Xiao, W.; Yang, L.; et al. Systems Pharmacology to Decipher the Combinational Anti-Migraine Effects of Tianshu Formula. *Drug Des. Dev. Ther.* 2018, 12, 1865. [CrossRef] [PubMed]

30. Bader, G.D.; Hogue, C.W.V. An Automated Method for Finding Molecular Complexes in Large Protein Interaction Networks. *BMC Bioinform.* 2003, 4, 2. [CrossRef] [PubMed]

31. Jones, G.; Willett, P.; Glen, R.C.; Leach, A.R.; Taylor, R. Development and Validation of a Genetic Algorithm for Flexible Docking. *J. Mol. Biol.* 1997, 267, 727–748. [CrossRef] [PubMed]
36. Feng, S.; He, X.; Zhong, P.; Zhao, J.; Huang, C.; Hu, Z. A Metabolism-Based Synergy for Total Coumarin Extract of Radix Angelicae Dahuricae and Ligustriazine on Migraine Treatment in Rats. *Molecules* **2018**, *23*, 1004. [CrossRef]

37. Deng, M.; Xie, L.; Zhong, L.; Liao, Y.; Liu, L.; Li, X. Imperatorin: A Review of Its Pharmacology, Toxicity and Pharmacokinetics. *Eur. J. Pharmacol.* **2020**, *879*, 173124. [CrossRef]

38. Chen, Y.; Wang, S.; Wang, Y. Role of Herbal Medicine for Prevention and Treatment of Migraine. *Phytother. Res.* **2022**, *36*, 730–760. [CrossRef]

39. Ma, Z.; Bai, L. The Anti-Inflammatory Effect of Z-Ligustilide in Experimental Ovariectomized Osteopenic Rats. *Inflammation* **2012**, *35*, 1793–1797. [CrossRef]

40. Shen, L.; Lin, X.; Hong, Y.; Liang, S.; Yuan, Y.; Feng, Y.; Xu, D.; Ruan, K. Study on HPLC Characteristic Fingerprint of Active Components of Dachuanxiong Fang in Plasma and Cerebrospinal Fluid. *China J. Chin. Mater. Med.* **2012**, *37*, 2017–2021.

41. Tronvik, E.; Stovner, L.J.; Bovim, G.; White, L.R.; Gladwin, A.J.; Owen, K.; Schrader, H. Angiotensin-Converting Enzyme Gene Insertion/Deletion Polymorphism in Migraine Patients. *BMC Neurol.* **2008**, *8*, 4. [CrossRef]

42. Jacobs, B.; Dussor, G. Neurovascular Contributions to Migraine: Moving beyond Vasodilation. *Neuroscience* **2016**, *338*, 130. [CrossRef]

43. Buzzi, M.G.; Moskowitz, M.A. The Pathophysiology of Migraine: Year 2005. *J. Headache Pain* **2005**, *6*, 105–111. [CrossRef]

44. Blacque, O.E.; Leroux, M.R. Bardet-Biedl Syndrome: An Emerging Pathomechanism of Intracellular Transport. *Cell. Mol. Life Sci.* **2006**, *63*, 2145–2161. [CrossRef]

45. Durham, P.L.; Russo, A.F. Stimulation of the Calcitonin Gene-Related Peptide Enhancer by Mitogen-Activated Protein Kinases and Repression by an Antimigraine Drug in Trigeminal Ganglia Neurons. *J. Neurosci.* **2003**, *23*, 807–815. [CrossRef]

46. Della Vedova, C.; Cathcart, S.; Dohnalek, A.; Lee, V.; Hutchinson, M.R.; Immink, M.A.; Hayball, J. Peripheral Interleukin-18 Levels Are Elevated in Chronic Tension-Type Headache Patients. *Pain Res. Manag.* **2013**, *19*, 301–306. [CrossRef]

47. Oliveira, A.B.; Bachi, A.L.L.; Ribeiro, R.T.; Mello, M.T.; Tufik, S.; Peres, M.F.P. Unbalanced Plasma TNF-α and IL-12/IL-10 Profile in Women with Migraine Is Associated with Psychological and Physiological Outcomes. *J. Neuroimmunol.* **2017**, *313*, 138–144. [CrossRef]

48. De Kloet, E.R.; Vreugdenhil, E.; Oitzl, M.S.; Joëls, M.; Joëls, J. Brain Corticosteroid Receptor Balance in Health and Disease. *Endocr. Rev.* **1998**, *19*, 269–301.

49. Pujols, L.; Mullol, J.; Roca-Ferrer, J.; Torrego, A.; Xaubet, A.; Cedillos, J.A.; Picado, C. Expression of Glucocorticoid Receptor α and β Isomers in Human Cells and Tissues. *Am. J. Physiol.-Cell Physiol.* **2002**, *283*, 1324–1331. [CrossRef]

50. Reuter, U.; Chiarugi, A.; Bolay, H.; Moskowitz, M.A. Nuclear Factor-KappaB as a Molecular Target for Migraine Therapy. *Ann. Neurol.* **2002**, *51*, 507–516. [CrossRef]

51. Shabab, T.; Khanabdali, R.; Moghadmtousi, S.Z.; Kadir, H.A.; Mohan, G. Neuroinflammation Pathways: A General Review. *Int. J. Neurosci.* **2012**, *127*, 624–633. [CrossRef]

52. Goadsby, P.J. Can We Develop Neuurally Acting Drugs for the Treatment of Migraine? *Nat. Rev. Drug Discov.* **2005**, *4*, 741–750. [CrossRef]

53. Liu, Y.Y.; Jiao, Z.Y.; Li, W.; Tian, Q. PI3K/AKT Signaling Pathway Activation in a Rat Model of Migraine. *Pain Res. Manag.* **2013**, *19*, 807–815. [CrossRef]

54. Hoffmann, J.; Charles, A. Glutamate and Its Receptors as Therapeutic Targets for Migraine. *Neurotherapeutics* **2018**, *15*, 361–370. [CrossRef] [PubMed]

55. Noble, E.P. D2 Dopamine Receptor Gene in Psychiatric and Neurologic Disorders and Its Phenotypes. *Am. J. Med. Genet. B, Neuropsychiatr. Genet.* **2003**, *116B*, 103–125. [CrossRef] [PubMed]

56. Marmura, M.J. Use of Dopamine Antagonists in Treatment of Migraine. *Curr. Treat. Options Neurol.* **2012**, *14*, 27–35. [CrossRef]

57. Hamel, E. Serotonin and Migraine: Biology and Clinical Implications. *Cephalalgia* **2007**, *27*, 1293–1300. [CrossRef] [PubMed]

58. Khan, J.; Al Asoom, L.I.; Al Sunni, A.; Rafique, N.; Latif, R.; Al Saif, S.; Almandil, N.B.; Almohazey, D.; AbdulAzeez, S.; Borgio, J.F. Genetics, Pathophysiology, Diagnosis, Treatment, Management, and Prevention of Migraine. *Biomed. Pharmacother.* **2021**, *139*, 111557. [CrossRef] [PubMed]

59. Kursun, O.; Yemisci, M.; van den Maagdenberg, A.M.J.M.; Karatas, H. Migraine and Neuroinflammation: The Inflammasome Perspective. *J. Headache Pain* **2021**, *22*, 1–13. [CrossRef] [PubMed]

60. Pusic, A.D.; Grinberg, Y.Y.; Mitchell, H.M.; Kraig, R.P. Modeling Neural Immune Signaling of Episodic and Chronic Migraine Using Spreading Depression In Vitro. *J. Vis. Exp.* **2011**, *52*, e2910. [CrossRef] [PubMed]

61. Eskandari, F.; Webster, J.L.; Sternberg, E.M. Neural Immune Pathways and Their Connection to Inflammatory Diseases. *Arthritis Res. Ther.* **2003**, *5*, 251. [CrossRef]

62. Duan, J.; Yu, Y.; Li, Y.; Li, Y.; Liu, H.; Jing, L.; Yang, M.; Wang, J.; Li, C.; Sun, Z. Low-Dose Exposure of Silica Nanoparticles Induces Cardiac Dysfunction via Neutrophil-Mediated Inflammation and Cardiac Contraction in Zebrafish Embryos. *Nanotoxicology* **2016**, *10*, 575–585. [CrossRef]

63. Xu, L.-M.; Li, J.-R.; Huang, Y.; Zhao, M.; Tang, X.; Wei, L. AutismKB: An Evidence-Based Knowledgebase of Autism Genetics. *Nucleic Acids Res.* **2012**, *40*, D1016–D1022. [CrossRef]

64. Smith, S.S. Female Sex Steroid Hormones: From Receptors to Networks to Performance—Actions on the Sensorimotor System. *Prog. Neurobiol.* **1994**, *44*, 55–86. [CrossRef]
90. Trott, O.; Olson, A.J. AutoDock Vina: Improving the Speed and Accuracy of Docking with a New Scoring Function, Efficient Optimization, and Multithreading. *J. Comput. Chem.* **2010**, *31*, 455–461. [CrossRef]

91. Oyebamiji, A.K.; Josiah, O.M.; Akintelu, S.A.; Adeoye, M.D.; Sabitu, B.O.; Latona, D.F.; Esan, A.O.; Soetan, E.A.; Semire, B. Dataset on Insightful Bio-Evaluation of 2-(Quinoline-4-Yloxy)Acetamide Analogues as Potential Anti-Mycobacterium Tuberculosis Catalase-Peroxidase Agents via In Silico Mechanisms. *Data Brief* **2021**, *38*, 107441. [CrossRef]

92. Saeed, M.; Shoaib, A.; Tasleem, M.; Alabdallah, N.M.; Alam, M.J.; El Asmar, Z.; Jamal, Q.M.S.; Bardakci, F.; Alqahtani, S.S.; Ansari, I.A.; et al. Assessment of Antidiabetic Activity of the Shikonin by Allosteric Inhibition of Protein-Tyrosine Phosphatase 1B (PTP1B) Using State of Art: An In Silico and In Vitro Tactics. *Molecules* **2021**, *26*, 3996. [CrossRef]