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Systems Analysis of Antihypertensive Effects of *Chuanxiong Rhizoma*

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ABSTRACT

We performed a systems pharmacological analysis of the components of *Chuanxiong Rhizoma* (CR) in particular regarding its antihypertensive effects, as the herb can be used to prevent and treat vascular diseases. Using the Traditional Chinese Medicine Systems Pharmacology database and analysis platform, 214 potential active compounds in CR were selected, and a network between the compounds with antihypertensive effects and their target genes and diseases was analysed. We evaluated 23 active compounds on the basis of their drug similarity, oral bioavailability, and Caco-2 permeability to determine their characteristics of drug absorption and distribution, metabolism, and excretion. Forty-one target genes identified using the UniProt Database had effects on 46 biological processes, identified using the David 6.8 Gene Functional Classification Tool. ADRA2A, ADRA2B, ADRB1, ADRB2, and NOS3 genes were involved in blood pressure regulation; (z)-ligustilide, 4-hydroxy-3-butylphthalide, methyl 2-pentanoylbenzoate, 4,7-dihydroxy-3-butylphthalide, 3-butylidene-7-hydroxyphthalide, sinapic acid, and L-bornyl acetate contained in CR and senkyunolide-D were identified as the related active compounds. These results may be used to develop prevention and treatment strategies for hypertension, but should be verified in future studies.

KEYWORDS: *Chuanxiong Rhizoma*, antihypertensive compounds, systems pharmacological analysis, TCMSP

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INTRODUCTION

Hypertension is a risk factor for cardiovascular diseases, such as heart failure, stroke, and chronic kidney disease. It contributes to the premature death of a quarter of men and one-fifth of women worldwide, resulting in over 1 billion deaths. In particular, in Europe and Central Asia, it accounts for one-third of deaths, and is considered a major risk factor for preventable death[1,2]. Therefore, maintaining a normal blood pressure is important to reduce the incidence and mortality of various diseases. Several studies have reported that poor blood pressure control is a major public health challenge worldwide[3]. Although anti-hypertensive medications are commonly used, almost 40% of hypertensive patients in developed countries do not have adequate blood pressure control[4].

Hypertension is primarily treated with thiazide diuretics, calcium channel blockers, angiotensin-converting enzyme (ACE) inhibitors, and angiotensin receptor blockers, either alone or in combination, depending on the clinical condition[5]. Despite using anti-hypertensive medications, the treatment and control rates are 48% and 38.6%, respectively, in China, Europe, and Latin America, and the control rate is 63% in South Korea. In reality, antihypertensive drugs have insufficient efficacy to reach blood pressure targets[6,7]. In addition, they are associated with various side effects, such as asthma, headache, and hyperkalemia. To overcome these limitations, antihypertensive vaccines and gene therapy are being used to improve current antihypertensive drugs and to develop new drugs with different mechanisms of action[8].

Herbal medicines are developed from natural substances and have fewer side effects compared to chemical drugs. Therefore, in recent years, the interest in herbal extracts has been increasing. However, herbal medicines have complex mechanisms of action and studies of herbal medicines need to consider several variables. Therefore, research on herbal medicines has several limitations. In this study, we used network-based systems pharmacological analysis to overcome these limitations. We developed a new strategy for controlling blood pressure by examining the antihypertensive effects of the main components of herbal medicines.

Chuanxiong Rhizoma (CR), the dried rhizome of *Ligusticum chuanxiong* Hort, belongs to the Umbelliferae family. In oriental medicine, it is mainly used to sooth the liver, relieve depression, remove dampness, reduce blood circulation pain, and reduce wound swelling and drainage. In addition, it improves blood circulation, induces vasodilation by its effect on the cardiovascular system, and inhibits vasoconstriction[9]. In a recent study, CR extract characteristically inhibited

retinal neovascularization, indicating that it is a potential therapeutic agent for ischemic retinopathy[10]. It may also be a potential anticancer drug due to its effect on a protein involved in the growth of human oral cancers[11].

CR contains various active compounds. However, studies have not evaluated these compounds because of methodological limitations that complicate the study of multi-component, complex mechanisms of action. In addition, research has not investigated the target genes related to the active components of CR. In this study, we conducted a network-based pharmacological analysis of compounds for blood pressure control and target genes related to CR. We also predicted the biological actions of the genes and their effects on blood pressure control. On the basis of bioinformatics data, we suggest that CR is a potential treatment of hypertension.

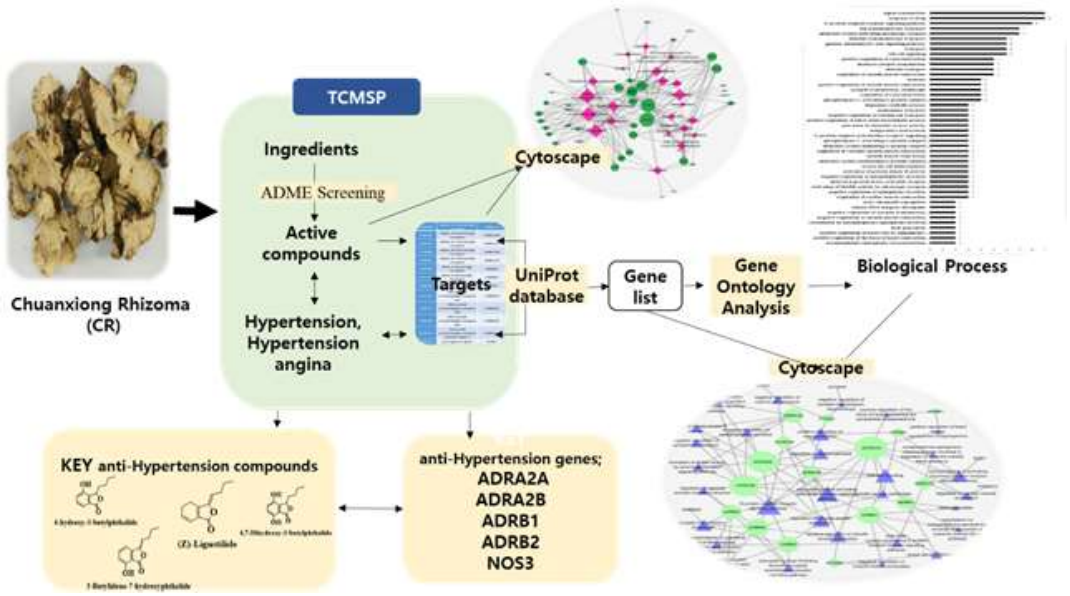


Figure 1. Workflow for the network-based systems pharmacological analysis of *Chuanxiong Rhizoma*, including active compound screening, target fishing, gene ontology analysis, and network construction.

MATERIALS AND METHODS

Identification of active compounds

The active ingredients of CR were screened using the Traditional Chinese Medicine Systems Pharmacology (TCMSP, https://tcmsp-e.com/) Database Analysis Platform[12]. The criteria for physiological activity (oral bioavailability, OB), drug likeness (DL), and intestinal absorption (Caco-

2 permeability, Caco-2) were used to screen the compounds, based on the properties of drug absorption, distribution, metabolism, and excretion. The screening values were set at OB of ≥ 50%, Caco-2 of ≥ –0.4, and DL of ≥ 0.05. In addition to the active compounds screened by the aforementioned three scales, sedanoic acid, a representative ingredient of CR, was also included in the analysis. Figure 1 summarizes the workflow of this study.

Table 1. Active compounds of *Chuanxiong Rhizoma*

Molecule Name	OB (%)	Caco-2	DL
()-Aromadendrene	55.74	1.81	0.1
(+)-ALPHA-FUNEBRENE	52.87	1.79	0.1
(Z)-Ligustilide	53.72	1.3	0.07
1-Acetyl-beta-carboline	67.12	1.18	0.13
1(3H)-Isobenzofuranone, 3-butyl-3a,4,5,6-tetrahydro-, cis-(-)-	65.03	1.25	0.07
1H-Cycloprop(e)azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, (1aR-(1aalpha,4aalpha,7beta,7abeta,7balpha))-	82.33	1.37	0.12
3-Butylidene-7-hydroxyphthalide	62.68	1	0.08
4-Hydroxy-3-butylphthalide	70.31	0.9	0.08
4,7-Dihydroxy-3-butylphthalide	106.09	0.69	0.1
49070_FLUKA	85.51	1.29	0.12
Aromadendrene oxide 2	65.1	1.56	0.14
Carotol	149.03	1.46	0.09
Cedrene	51.14	1.82	0.11
cis-Ligustilide	51.3	1.3	0.07
Cnidilide	77.55	1.21	0.07
L-Bornyl acetate	65.52	1.29	0.08
methyl 2-pentanoylbenzoate	69.28	0.91	0.07
Neocnidilide	83.83	1.23	0.07
Perlolyrine	65.95	0.88	0.27
Senkyunolide-D	79.13	0.12	0.1
Senkyunolide-K	61.75	0.52	0.08
Sinapic acid	64.15	0.48	0.08
Sedanoic-acid	44.69	0.37	0.06

Target protein collection and biological process analysis

Genetic information on the target protein of the compounds was obtained from the UniProt database (http://www.uniprot.org). To identify the biological process related to the secured gene, DAVID 6.8 Gene Functional Classification Tool (https://david.ncifcrf.gov/) was used. The p-value was set to < 0.01 and corrected using the Benjamini-Hochberg method.

Network analysis of the identified targets

Cytoscape 3.7.0 (https://cytoscape.org/) was used to construct a network of active ingredients of CR and target genes (compound-target network), as well as of target and biological processes (process–target network).

RESULTS

Identification of active compounds

The TCMSP analysis identified 214 potential active compounds. The target information was collected for sedanoic acid and 23 active compounds, of which 22 fulfilled the set conditions (OB ≥ 50%; Caco-2 ≥ –0.4; DL ≥ 0.05) (Table 1).

Target protein collection and biometabolic analysis

After excluding duplicates, 41 genes associated with 23 compounds were collected (Table 2). Using the Gene Functional Classification Tool, we identified 46 biological processes associated with the aggregated genes (Figures 2 and 3B).

Table 2. Target proteins of *Chuanxiong Rhizoma*

Target	Gene name	Target	Gene name
Alcohol dehydrogenase 1C	ADH1C	Glutamate receptor 2	GRIA2
Alpha-1A adrenergic receptor	ADRA1A	Heat shock protein HSP 90	HSP90AB1
Alpha-1B adrenergic receptor	ADRA1B	5-hydroxytryptamine 2A receptor	HTR2A
Alpha-2A adrenergic receptor	ADRA2A	Leukotriene A-4 hydrolase	LTA4H
Alpha-2B adrenergic receptor	ADRA2B	Amine oxidase [flavin-containing] A	MAOA
Alpha-2C adrenergic receptor	ADRA2C	Amine oxidase [flavin-containing] B	MAOB
Beta-1 adrenergic receptor	ADRB1	Nuclear receptor coactivator 2	NCOA2
Beta-2 adrenergic receptor	ADRB2	Nitric oxide synthase, inducible	NOS2
Choline O-acetyltransferase	CHAT	Nitric-oxide synthase, endothelial	NOS3
Muscarinic acetylcholine receptor M1	CHRM1	CGMP-inhibited 3',5'-cyclic phosphodiesterase A	PDE3A
Muscarinic acetylcholine receptor M2	CHRM2	cAMP-dependent protein kinase inhibitor alpha	PKIA
Muscarinic acetylcholine receptor M3	CHRM3	mRNA of PKA Catalytic Subunit C-alpha	PRKACA
Neuronal acetylcholine receptor subunit alpha-2	CHRNA2	Prostaglandin G/H synthase 1	PTGS1
Dopamine D1 receptor	DRD1	Prostaglandin G/H synthase 2	PTGS2
Estrogen receptor	ESR1	Retinoic acid receptor RXR-alpha	RXRA
Gamma-aminobutyric acid receptor subunit alpha-1	GABRA1	Sodium channel protein type 5 subunit alpha	SCN5A
Gamma-aminobutyric-acid receptor alpha-2 subunit	GABRA2	Sodium-dependent noradrenaline transporter	SLC6A2
Gamma-aminobutyric-acid receptor alpha-3 subunit	GABRA3	Sodium-dependent dopamine transporter	SLC6A3
Gamma-aminobutyric-acid receptor subunit alpha-4	GABRA4	Sodium-dependent serotonin transporter	SLC6A4
Gamma-aminobutyric-acid receptor alpha-5 subunit	GABRA5	DNA topoisomerase II	TOP2A
Gamma-aminobutyric-acid receptor subunit alpha-6	GABRA6		

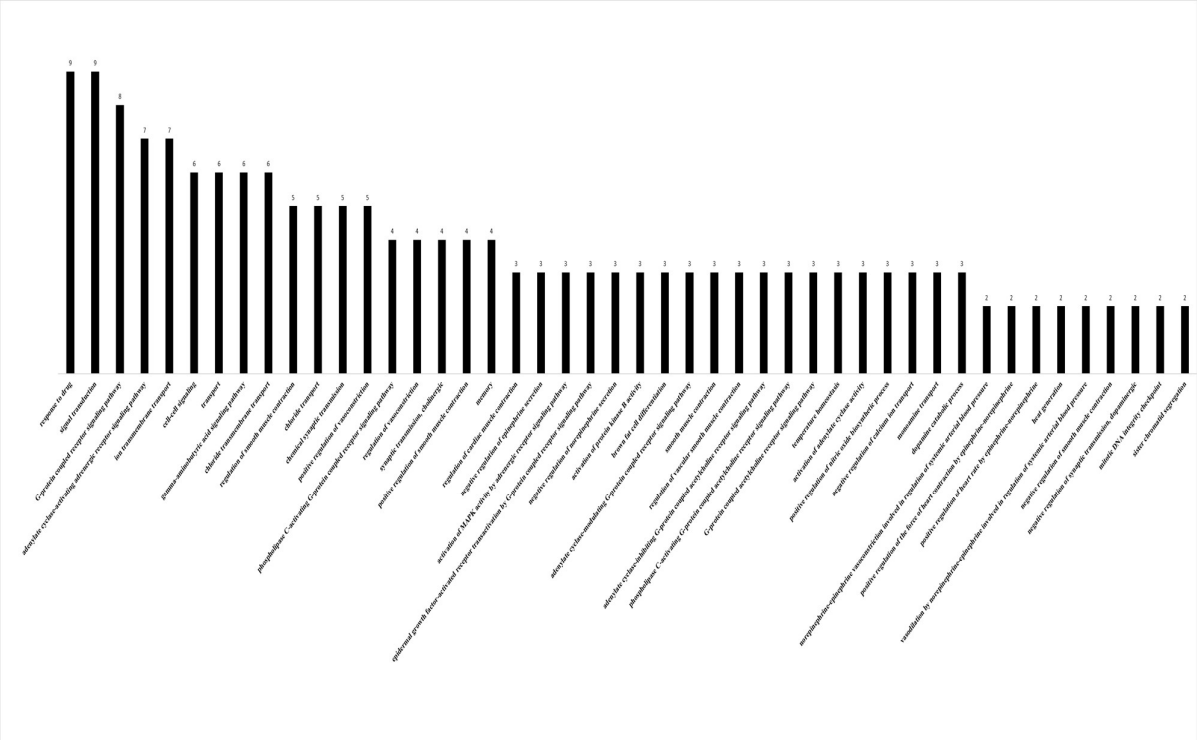
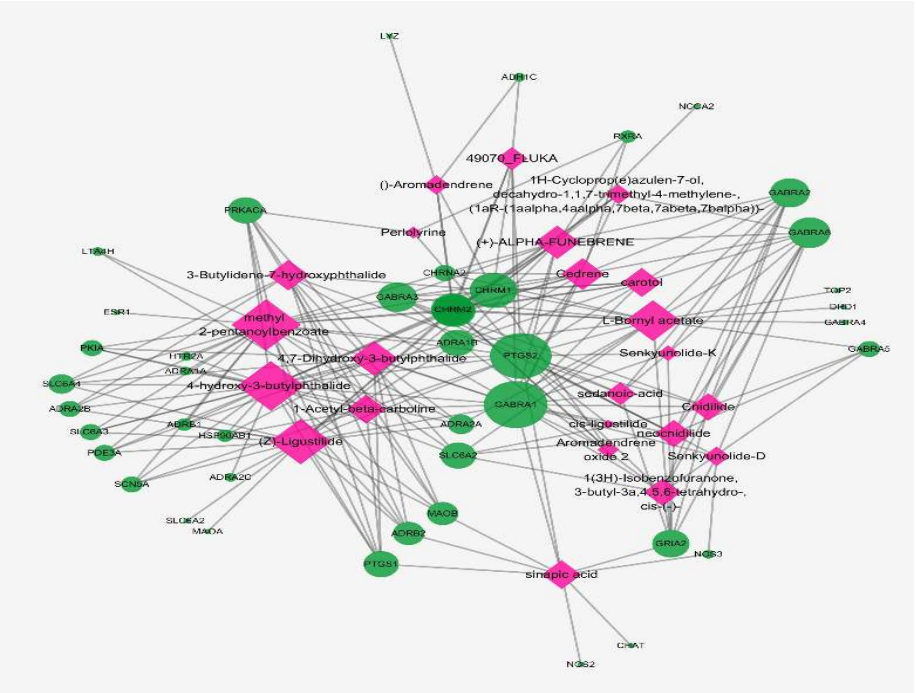


Figure 2: Biological processes (n = 46) elicited from the gene ontology analysis of the Chuanxiong Rhizoma target genes. The values represent the number of related genes.

A



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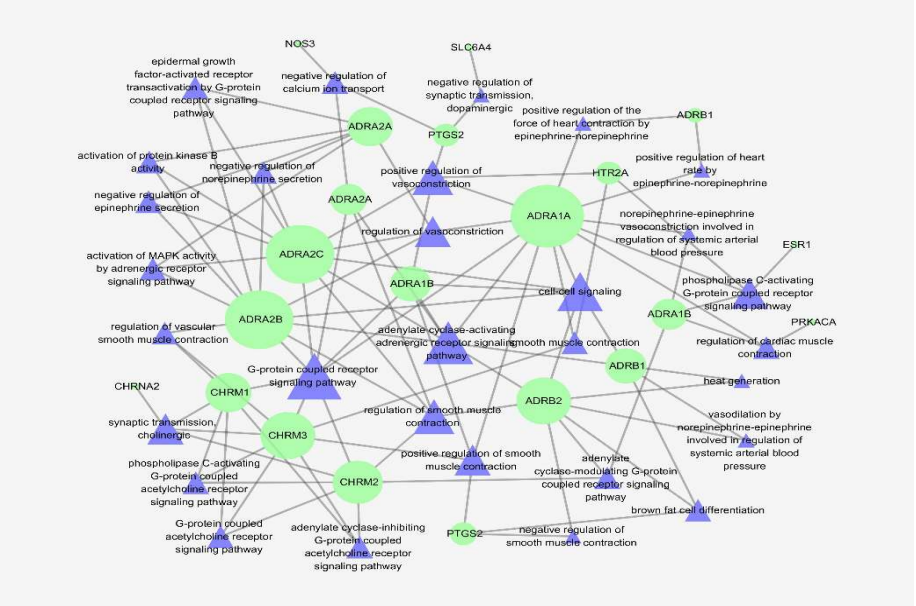

Figure 3: The network of *Chuanxiong Rhizoma*. (A) Compound-target network; diamond-shaped purple nodes: active compounds, green circular nodes: target genes. (B) Process-target network; triangle-shaped indigo node: biological process, circular emerald node: target gene. Node size reflects the strength of effects.

We searched for 'hypertension' and 'hypertension, angina' in the TCMSP, and identified 25

genes related to hypertension. Among the 25 genes identified from TCMSP and 41 identified previously, 5 were selected (Figure 4).

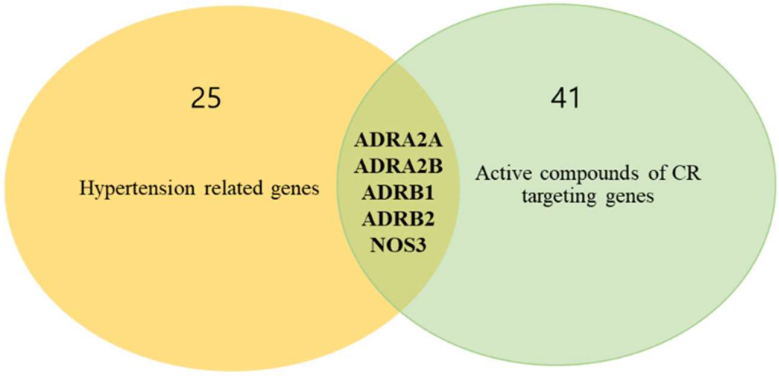

Figure 4: Genes associated with both hypertension and *Chuanxiong Rhizoma* compound targets.

These genes were alpha-2A adrenergic receptor (ADRA2A), alpha-2B adrenergic receptor (ADRA2B), beta-1 adrenergic receptor (ADRB1), beta-2 adrenergic receptor (ADRB2), and nitric-oxide synthase, endothelial (NOS3). These genes were involved in 22 out of the 46 previously identified biological processes that had an antihypertensive action (Table 3).

Table 3. Antihypertensive effects of the biological processes and related genes.

Biological Process	**Genes**								
Signal transduction	GRIA2	CHRM3	CHRM1	GABRA6	GABRA5	ADRA2A	ESR1	ADRA1A	CHRNA2
G-protein coupled receptor signaling pathway	CHRM3	CHRM2	CHRM1	ADRA2A	ADRA1B	ADRA1A	ADRA2C	ADRA2B	
Adenylate cyclase-activating adrenergic receptor signaling pathway	ADRB2	ADRB1	ADRA2A	ADRA1B	ADRA1A	ADRA2C	ADRA2B		
Cell-cell signaling	ADRB2	ADRB1	ADRA1B	ADRA1A	ADRA2C	ADRA2B			
Regulation of smooth muscle contraction	ADRB2	CHRM2	ADRA2A	ADRA2C	ADRA2B				
Regulation of vasoconstriction	ADRA2A	ADRA1A	ADRA2C	ADRA2B					
Negative regulation of epinephrine secretion	ADRA2A	ADRA2C	ADRA2B						
Activation of MAPK activity by adrenergic receptor signaling pathway	ADRA2A	ADRA2C	ADRA2B						

Epidermal growth factor-activated receptor transactivation by G-protein coupled receptor signaling pathway	ADRA2A	ADRA2C	ADRA2B						
Negative regulation of norepinephrine secretion	ADRA2A	ADRA2C	ADRA2B						
Activation of protein kinase B activity	ADRA2A	ADRA2C	ADRA2B						
Brown fat cell differentiation	ADRB2	ADRB1	PTGS2						
Adenylate cyclase-modulating G-protein coupled receptor signaling pathway	ADRB2	CHRM2	ADRA1B						
Regulation of vascular smooth muscle contraction	CHRM3	CHRM1	ADRA2B						
Temperature homeostasis	DRD1	ADRB1	HTR2A						
Activation of adenylate cyclase activity	DRD1	ADRB2	ADRB1						
Negative regulation of calcium ion transport	PTGS2	ADRA2A	NOS3						
Positive regulation of the force of heart contraction by epinephrine-norepinephrine	ADRB1	ADRA1A							
Positive regulation of heart rate by epinephrine-norepinephrine	ADRB1	ADRA1A							
Heat generation	ADRB2	ADRB1							
Vasodilation by norepinephrine-epinephrine involved in regulation of systemic arterial blood pressure	ADRB2	ADRB1							
Negative regulation of smooth muscle contraction	ADRB2	PTGS2							

Eight active compounds targeted these genes, including (Z)-ligustilide, 4-hydroxy-3-butylphthalide, methyl 2-pentanoylbenzoate, 4,7-dihydroxy-3-butylphthalide, 3-butylylidene-7-hydroxyphthaide, sinapic acid, L-bornyl acetate, and senkyunolide-D (Figure 5).

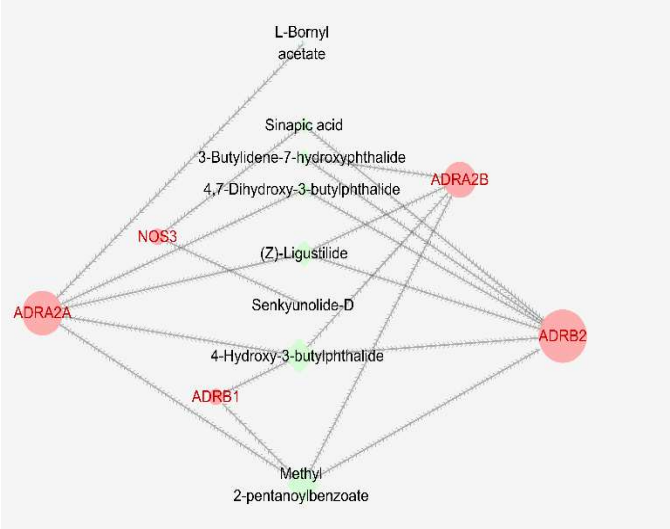

Figure 5: Hypertension-related key genes and compounds of *Chuanxiong Rhizoma*. Diamond-shaped green nodes represent the compounds and pink circular nodes represent the genes. Node size reflects the strength of effects.

In summary, 22 active ingredients and sedanoic acid satisfied the set conditions (OB ≥ 50%; Caco-2 ≥ –0.4; DL ≥ 0.05), and targeted 41 genes (Tables 1 and 2). These genes were involved in 46 biological processes (Figures 2 and 3B). Of these, 22 biological processes were associated with antihypertensive effects (Table 3).

Among the 25 hypertension-related genes from TCMSP and 41 genes targeted by CR active compounds, common hypertension-related genes were ADRA2A, ADRA2B, ADRB1, ADRB2, and NO3. These genes were targeted by (Z)-ligustilide, 4-hydroxy-3butylphthalide, methyl 2-pentanoylbenzoate, 4,7-dihydroxy-3-butylphthalide, 3-butylidene-7-hydroxyphthalide, sinapic acid, L-bornyl acetate, and senkyunolide-D (Figures 4 and 5).

Based on these results, we conclude that the main active ingredients of CR play an important role in blood pressure control. The components of CR may be promising antihypertensive agents or adjuvants.

DISCUSSION

The TCMSP database and analysis platform is a systems pharmacological platform for herbal analysis, which allows the identification of correlations between drugs, targets, and diseases. The database contains chemical-target and drug-target networks, and pharmacokinetic properties of natural compounds, such as oral bioavailability, drug-likeness, and intestinal epithelial permeability. These properties can be set to the desired conditions. Therefore, candidate compounds for herbal medicines can be searched at the systems network level. The 23 active compounds identified in this

study targeted 41 genes and were involved in 46 biological processes. Of these, 8 active compounds, 5 genes, and 22 biological processes were associated with antihypertensive effects. This suggests that the active compounds of CR may be effective for treating hypertension.

The representative active compounds involved in the antihypertensive effect were (Z)-ligustilide, 3-butylidene-7-hydroxyphthalide, 4-hydroxy-3-butylphthalide, methyl 2-pentanoylbenzoate, 4,7-dihydroxy-3-butylphthalide, 3-butylidene-7-hydroxyphthalide, L-bornyl acetate, and senkyunolide-D.

Ligustilide (3-butylidene-4,5-dihydroisodenzofuranone), a phthalide derivative that exists in nature, is an active component of herbal medicines such as angelica and CR. (Z)-ligustilide has various pharmacological effects, such as kidney protection, vascular relaxation, neuroprotection, analgesia, and antispasmodic, anti-cancer, anti-cell death, anti-inflammatory, and antioxidant effects[13]. It inhibits voltage-dependent calcium channels and receptor-mediated calcium channels, thereby inhibiting the influx of extracellular Ca^{2+} and relaxing the blood vessels[14].

In this study, we identified 3-butylidene-7-hydroxyphthalide, 4-hydroxy-3-butylphthalide, methyl 2-pentanoylbenzoate, and 4,7-dihydroxy-3-butylphthalide as the major active compounds involved in blood pressure control. All of the aforementioned compounds are alkylphthalides, but their mechanisms of action are not clear. However, they probably act on the alpha-2 adrenergic receptor, which inhibits norepinephrine secretion through negative feedback, and beta-2 adrenergic receptor, which relaxes vascular smooth muscle. In addition, butyldienphtalide, which has a structure similar to that of the abovementioned four compounds, inhibits L-type voltage-operated calcium channels and TP-ROCC (receptor-operated Ca^{2+} channels), which have pre-systolic effects that reduce the blood pressure. Therefore, the aforementioned four active compounds are closely linked to antihypertensive effects[15].

Bornyl acetate is commonly used in food additives, fragrances, and plasticizers in perfumes. It is a main active compound of CR. Bornyl acetate also has antioxidant and anti-inflammatory effects[16]. L-bornyl acetate causes vascular relaxation by blocking adrenergic alpha1-recpeptors[17], and reduces the systolic, diastolic, and average blood pressures *in vivo*. Computer-modelled analysis of the mechanism of action of the active substance of bornyl acetate showed that it inhibits the activity of ACE proteins by strong hydrogen binding[18].

Sinapic acid is a naturally occurring small hydroxylic acid from the phenylpropanoid family. It is

widely spread across the plant system and is commonly found in rye, fruits, and vegetables. Sinapic acid promotes relaxation of the vascular smooth muscles by protecting endothelial cells from oxidative stress and promoting nitric oxide production[19].

Senkyunolide and ligustilide have the highest concentrations in CR[20]. Senkyunolide has several subtypes. In this study, we evaluated senkyunolid-D as a potential active compound. The role of senkyunolide-D is not yet clear, but previous studies have suggested that senkyunolide-D targets NOS3. Senkyunolide-A, which has a structure similar to senkyunolid-D, mitigates vascular contraction. Therefore, senkyunolide-D has a close association with antihypertensive effects[21].

The active ingredients selected in this study had antihypertensive effects, which is consistent with previous studies. Highly relevant targets for hypertensive active compounds of CR were ADRA2A, ADRA2B, ADRB1, ADRB2, and NOS3. ADRA2A and ADRA2B encode for α2A and α2B receptors, which are subtypes of the alpha-2 adrenergic receptor. The alpha-2 adrenergic receptor lowers blood pressure through negative feedback on the norepinephrine pre-synaptic receptor[22].

ADRB1 is a beta-1 adrenergic receptor that improves blood flow by increasing the heart rate, ECG rate, and myocardial relaxation rate[23]. ADRB2 is a beta-2 adrenergic receptor; epinephrine binds to ADRB2 to activate adenylate cyclase and increase cAMP, thereby causing vascular smooth muscle relaxation by inhibition of Ca^{2+} inflow through the L-type calcium channel into vascular smooth muscle[24]. NOS3 is a nitric-oxide synthase that promotes nitric oxide production from L-arginine, which stimulates guanylate cyclase to produces cGMP from GTP. Due to increased cGMP, myocyte Ca^{2+} enters into the endoplasmic reticulum or exits the cells, resulting in muscle relaxation and reduced blood pressure[25].

In this study, we used systems pharmacological analysis to analyse the components of the active compounds of CR with antihypertensive effects, and target genes and biological processes thereof. The use of network-based systems to analyse the pharmacological action of drugs reduces the time, cost, and effort associated with the search process. In addition, multiple potential drugs can simultaneously be evaluated to identify those with favourable multi-target properties for a disease. The data obtained from this study are expected to be useful for developing strategies to treat hypertension.

CONCLUSION

We analysed the antihypertensive effects of active compounds of CR, as well as their target proteins and genes, using systems pharmacological analysis. We identified the components of CR using the TCMSP database and analysis platform. This platform includes pharmacokinetic properties of compounds, target information, and related diseases, which allows an *in silico* approach to analysis at the network level. Using target gene information from the UniProt database and gene ontology analysis using the David 6.8 gene function classification tool, 23 active compounds were evaluated by adding 22 active ingredients and sedanoic acid (a representative ingredient of CR). We identified 41 genes related to the 23 active ingredients. These 41 genes were involved in 46 biological processes.

Five genes with antihypertensive effects were selected by comparing the identified 41 genes with 25 genes identified from the TCMSP as being related to hypertension. These genes were involved in 22 of the 46 biological processes related to antihypertensive effects. The major components that targeted the five selected genes included (Z)-ligustilide, 3-butylidene-7-hydroxyphthalide, 4-hydroxy-3-butylphthalide, methyl-2-pentanoylbenzoate, 4,7-dihydroxy-3-butylphthalide, 4,7-dihydroxy-3-3-butylidene-7-hydroxyphthalide, 4,7-dihydroxy-3sinapic acid, l-bornyl acetate, and senkyunolide-D.

Our results demonstrate a close relationship between the active compounds of CR, target genes, and related biological processes related to antihypertensive effects. These results will help in developing strategies for treating hypertension. However, further studies are required to validate our results.

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REFERENCES

1. WHO Team. “Hypertension” [Online]. 2020 [cited 2021 Jul 10]. Available from: URL: https://www.who.int/health-topics/hypertension#tab=tab_1.
2. Lawes CM, Vander HS, Rodgers A; International Society of Hypertension. Global burden of blood-pressure-related disease, 2001. Lancet. 2008; 371(9623): 1513-8.
3. Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of

hypertension: analysis of worldwide data. Lancet. 2005; 15-21: 365(9455):217-23.

4. M Wang. Coronary Artery Disease: Therapeutics and Drug Discovery. Springer. Cham; 2020.
5. Unger T, Borghi C, Charchar F, Khan NA, Poulter NR, Prabhakaran D, Ramirez A, Schlaich M, Stergiou GS, Tomaszewski M, Wainford RD, Williams B, Schutte AE. 2020 International Society of Hypertension Global Hypertension Practice Guidelines. Hypertension. 2020; 75(6):1334-1357.
6. Melgarejo JD, Maestre GE, Thijs L, et al. Prevalence, Treatment, and Control Rates of Conventional and Ambulatory Hypertension Across 10 Populations in 3 Continents. Hypertension. 2017;70(1):50-58.
7. Kim HC, Cho SMJ, Lee H, Lee HH, Baek J, Heo JE. Korean Society of Hypertension (KSH) – Hypertension Epidemiology Research Working Group. Korea hypertension fact sheet 2020: analysis of nationwide population-based data. Clin Hypertens. 2021; 27(1): 8
8. Cho MC. New strategies in the treatment of hypertension. The Korean Journal of Medicine 2009; 76(4): 409-415.
9. Park YC, Lee SD, Heo Y, Kim HS, Lee IS. Effects of Ligusticum Chuanxiang on blood circulation and oxidative stress. J of Society of Preventive Korean Medicine 2002:6(2):86–94.
10. Lee YM, Lee YR, Kim CS, Jo K, Sohn E, Kim JS, Kim J. Cnidium officinale extract and butylidenephthalide inhibits retinal neovascularization in vitro and in vivo. BMC Complement Altern Med. 2016; 16:231.
11. Lee KE, Shin JA, Hong IS, Cho NP, Cho SD. Effect of methanol extracts of Cnidium officinale Makino and Capsella bursa-pastoris on the apoptosis of HSC-2 human oral cancer cells. Exp Ther Med. 2013; 5(3): 789-792.
12. Ru J, Li P, Wang J, Zhou W, Li B, Huang C, Li P, Guo Z, Tao W, Yang Y, Xu X, Li Y, Wang Y, Yang L. TCMSP: a database of systems pharmacology for drug discovery from herbal medicines. J Cheminform. 2014; 6:13.
13. Xie Q, Zhang L, Xie L, Zheng Y, Liu K, Tang H, Liao Y, Li X. Z-ligustilide: A review of its pharmacokinetics and pharmacology. Phytother Res. 2020; 34(8):1966-1991.
14. Cao YX, Zhang W, He JY, He LC, Xu CB. Ligustilide induces vasodilatation via inhibiting voltage dependent calcium channel and receptor-mediated Ca2+ influx and release. Vascul Pharmacol. 2006; 45(3): 171-6.
15. Chan SS, Choi AO, Jones RL, Lin G. Mechanisms underlying the vasorelaxing effects of butylidenephthalide, an active constituent of Ligusticum chuanxiong, in rat isolated aorta. Eur J Pharmacol. 2006; 537(1-3): 111-7.

16. Yang L, Liu J, Li Y, Qi G. Bornyl acetate suppresses ox-LDL-induced attachment of THP-1 monocytes to endothelial cells. Biomed Pharmacother. 2018;103: 234-239.
17. Lim DY, Ki YW, Na GM, Kang MJ, Kim BC, Kim OM and Hong SP: Influence of Bornyl Acetate on Blood Pressure and Aortic Strips Contractility of the Rat. Biomolecules & Therapeutics. 2003; 11(2);119-125,
18. Noor N, Alamgeer -, Alyami BA, Alqarni AO, Alqahtani YS, Mahnashi MH, Akram M, Qasim S, Younis W, Ali E, Shamim R. Appraisal of hypotensive effect of natural monoterpene: Bornyl acetate and in-silico molecular docking analysis. Pak J Pharm Sci. 2021; 34(2(Supplementary)): 731-735.
19. Silambarasan T, Manivannan J, Krishna Priya M, Suganya N, Chatterjee S, Raja B. Sinapic acid prevents hypertension and cardiovascular remodeling in pharmacological model of nitric oxide inhibited rats. PLoS One. 2014; 9(12): e115682.
20. Kim KW, Jeong HJ, Chung GY, Sohn HJ, Oh SM, Kim SY, Nam SH, Park JH and Shim YE. Comparison on the Phthalides content of Cinidil Rhizoma and Angelicae Radix cultivated in Korea and China. Korean Journal of Plant Resources. 2001;14(1): 24~31.
21. Chan SS, Cheng TY, Lin G. Relaxation effects of ligustilide and senkyunolide A, two main constituents of Ligusticum chuanxiong, in rat isolated aorta. J Ethnopharmacol. 2007; 111(3): 677-80.
22. Kanagy NL. Alpha(2)-adrenergic receptor signalling in hypertension. Clin Sci (Lond). 2005; 109(5): 431-7.
23. Alhayek S, Preuss CV. "Beta 1 Receptors" [Online]. 2021 [cited 2021 Jul 22]. Available from: URL: https://www.ncbi.nlm.nih.gov/books/NBK532904/
24. Rang HP. Pharmacology. Edinburgh: Curchill Livingstone; 2003.
25. Chen K, Pittman RN, Popel AS. Nitric oxide in the vasculature: where does it come from and where does it go? A quantitative perspective. Antioxid Redox Signal. 2008; 10(7): 1185-98.

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