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Research Article

Network Pharmacology Approach to Elucidate Possible Action Mechanisms of *Hippophae Rhamnoides Linn* for Treating High Altitude Disease

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Abstract

Hippophae rhamnoides Linn (HRL) is used for the treatment of High-Altitude Sickness (HAS). However, its mechanism remains unclear. This study was aimed to identify the bioactive constituents and potential mechanism of HRL for treating HAS using network pharmacology and molecular docking. Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) was used to obtain the active constituents and targets of HRL. The related targets of HAS were searched from Gene Cards, OMIM, Drug bank, and PharmGkb database, thereby obtaining the targets of HRL against HAS. After merging HAS-related targets and active compound targets of HRL, the overlapping targets were recognized as candidate targets. PPI network was constructed by importing the gene ID of the candidate targets to the STRING database and the core targets were obtained by cytoscape 3.7.2, next, a compound-target network was constructed using Cytoscape 3.7.2 software. The Metascape online tool was used to perform gene ontology (GO) and Kyoto Encyclopedia Of Genes And Genome (KEGG) pathway enrichment analysis of overlapping targets. Finally, molecular docking were performed to assess the binding activities between the compounds and anti-HAS targets of HRL in treating HAS. The results showed that there were 33 active ingredients (16 key active ingredients) in HRL and 48 targets were screened out for HAS treatment. Network analysis indicated that core targets of main active components of HRL were target genes such as HIF-1A, VEGFA, NR3C1, MAPK14, CAT, AR, AKT1, TP53, which are involved in the regulation of pathways in cancer, fluid shear stress and atherosclerosis, HIF-1 signaling pathway, VEGF signaling pathway, complement and coagulation cascades, and so forth. The study revealed potential mechanism regarding the anti-hypoxia effect of active components in HRL by network pharmacology and molecular docking analyses.

Keywords: *Hippophae rhamnoides Linn* (HRL); High altitude sickness; Network pharmacology; Molecular docking; Quercetin

Abbreviations: BC: Betweenness Centrality; DC: Degree Centrality; DL: Drug-Likeness; EC: Eigenvector Centrality; GO: Gene Ontology; HAS: High Altitude Sickness; HRL: Hippophae Rhamnoides Linn; KEGG: Kyoto Encyclopedia of Genes and Genomes Database; LAC: Local Average Connectivity; MW: Molecular Weight; NC: Network Centrality; NCBI: National Center for Biotechnology Information; OB: Oral Bioavailability; PPI: Protein-Protein Interaction; TCM: Traditional Chinese Medicine; TTD: Therapeutic Target Database; TCMSP: Traditional Chinese Medicine System Pharmacology Database and Analysis Platform.

Introduction

Every year, large numbers of people are ascending to high altitudes for the purposes of pleasure and work. After ascent to high altitude (≥2500 m), the inability of the human body to adapt to the hypobaric and hypoxia environment can induce tissue hypoxia, then a series of high-altitude diseases (HAD) including Acute Mountain Sickness (AMS), High Altitude Pulmonary Edema (HAPE), and High-Altitude Cerebral Edema (HACE) would develop. These diseases can develop at any time from several hours to 5 days and can range in severity from mild with minimal effect on the planned travel itinerary to life-threatening illness [1, 2]. Therefore, it is needed to develop new drug of the treatment of HAS. Traditional Chinese Medicine (TCM), which embraces centuries of knowledge and practical experience, has been used to treat many complex diseases in China [3-5]. In the highlands of our country, TCM show great poten-

tial in the prevention and treatment of HAD because of their low price, rich resources and low side effect. Among them, *Hippophae Rhamnoides Linn*. (HRL) has been widely used in the treatment of HAS [6-7]. However, the underlying mechanisms of these anti-hypoxia effects remain unclear because of the complex compositions. Generally, the complex components in TCM exert their pharmacological effect through a multi-target and multi-pathway, which can hardly be elucidated by traditional methods. Recent developments in computational methods for screening digital compound libraries for multi-targeting drugs has resulted in a new branch of bioinformatics called network pharmacology. Network pharmacology is a integrity, synergy and dynamics analysis method based on disease, gene, protein target and drug interaction network. Up to now, it has been widely used to study TCM [8-12]. The purpose of the study is to screen the bioactive components in HRL and elucidate its molecular mechanisms in the treatment of HAS by network pharmacology. Finally, molecular docking were performed to validate the binding activities between the ingredients and anti-HAD targets of HRL.

Materials and Methods

Active compounds and corresponding target collection. Firstly, the chemical components of HRL were searched using TCMSP (http://tcmspw.com/tcmsp.php). Secondly, DL ≥ 0.18 and OB ≥ 30% were set as the thresholds to screen the active compounds via ADME analysis [13]. Thirdly, we screened the targets related to the active compounds using the TCMSP databases and obtained the Gene ID of the targets from the uniport (https://www.uniprot.org/) database.

Candidate target collection

The genes of targets associated with "high altitude sickness" and "high mountain sickness" disease names were collected through Gene Cards (https://www.genecards.org/), OMIM (https://www.omim.org/), Drug Bank database (https://www.drugbank.ca) and PharmGkb database (https://www.pharmgkb.org/). The Venny 2.1 (http://bioinfogp.cnb.csic.es/tools/venny/index.html) online tool was used to map the active ingredient target and the disease target of HAS and to draw Venn diagrams. After removing the duplicate genes, the overlapping genes of targets related to HAS were collected as the candidate targets.

Creating a PPI network

Common gene targets of HRL and HAS were imported into the STRING database (https://string-db.org/) to create a PPI network using the species qualified Homo sapiens, a confidence level of 0.9 and hidden disconnected nodes. Subsequently, the PPI network was visualized and further analyzed using Cytoscape software 3.7.2. The topological property of nodes in the interaction network were assessed by calculating six parameters with the CytoNCA plug-in: The six parameters which were "Degree Centrality (DC)," "Betweenness Centrality (BC)," "Closeness Centrality (CC)," "Eigenvector Centrality (EC)," "Network Centrality (NC)," and "Local Average Connectivity (LAC)" were used to measure the importance of nodes in the network. A node with high DC, BC, CC, EC, NC and LAC values means that it plays a highly important role in the network. Based on the results of topological property analysis of the above PPI, targets above the median were selected as the core targets.

GO and KEGG pathway enrichment

Potential targets were screened by the KEGG pathway and GO analysis (biological process, molecular function and cellular component) using the Metascape (https://metascape.org/) database. P-values ($P<0.05$) were considered as statistically significant, with a smaller P-value indicating a more significant correlation. The results of the analysis were selected the top 10 items with the highest enrichment and displayed them in the form of bubble graphs using website (http://www.bioinformatics. com.cn/).

Construction of the disease-target-compound network

To analyze the association among the candidate targets, the active compounds, a component-target network was obtained using Cytoscape 3.7.2 software. In the network, the nodes of different colors and shapes represented different targets and active compounds. Then, the core compounds were obtained through the component-target network.

Molecular docking

Core targets were obtained from the PPI network for molecular docking. With the help of the NCBI database (https://www.ncbi.nlm. nih.gov/pubmed/), the 2D structures of the ligand molecules were obtained and tranformated to 3D structures by chemoffice software and stored as a Mol2 file. The PDB-ID of the core targets ware also accessed. First, ligands for docking can be prepared through PyMOL selections including water deletion and the extraction of original ligands, ions and solvents. Then proteins and ligands were saved through Autodock Tools in PDBQT formats and were docked by perl software. Vina software was used to validate network pharmacology by molecular docking. Affinity (kcal/mol) is described by the minimum free energy which represents the degree of docking coincidence of molecules. The lower the minimum free energy is, the better the binding of ligands to receptor proteins is.

Results and Discussion

Active compounds and targets HRL

After screening was performed for OB ≥ 30% and DL ≥ 0.18, 33 active compounds and 423 related targets of the active compounds were searched via the TCMSP databases. Detailed information about these ingredients is provided in Table 1. Next, these targets were transformed into gene names and gene IDs via the Uniprot database, null and repetitive targets were deleted, and 182 effective active ingredient targets were obtained.

Table 1:33 compounds from HRL and corresponding OB and DL.

Mol ID	Molecular name	MW	OB	DL
MOL001004	pelargonidin	271.3	38	0.2
MOL010211	14,15-dimethyl-alpha-sitosterol	454.9	43.1	0.8
MOL010212	14--dimethyl-alpha-sitosterol	440.8	43.5	0.8
mol010227	canthaxanthine	564.9	41.6	0.6
mol010228	carotenoid	569	40.8	0.6
mol010230	ST5330591	490.8	48.1	0.8
mol010232	cislycopene	537	45.5	0.5
mol010234	delta-carotene	537	31.8	0.6
mol010240	ergosta-7-en-3-beta-ol	473	38.8	0.8
mol010241	ergostenol	400.8	35.4	0.7
mol010247	(2R, 6S,7aR)-2-[(1E,3E,5E,7E,9E,11E,13E,15E)-16-[(1R,4R)-4-hydroxy-2,6,6-trimethyl-1-cyclohex-2-enyl]-1,5,10,14-tetramethylhexadeca-1,3,5,7,9,11,13,15-octaenyl]-2,4,4,7a-tetramethyl-6,7-dihydro-5H-benzofuran-6-ol	590	57.9	0.5
mol010248	gamma-carotene	537	31	0.6
mol001979	LAN	426.8	42.1	0.8
mol010267	LYC	537	32.6	0.5
mol010283	ZINC03831331	450.8	47.6	0.7
mol001420	ZINC04073977	412.8	38	0.8
mol001494	mandenol	308.6	42	0.2
mol001510	24-epicampesterol	400.8	37.6	0.7
mol002268	rhein	284.2	47.1	0.3
mol002588	(3S,5R,10S,13R,14R,17R)-17-[(1R)-1,5-dimethyl-4-methylenehexyl]-4,4,10,13,14-pentamethyl-2,3,5,6,7,11,12,15,16,17-decahydro-1H-cyclopenta[a]phenanthren-3-ol	440.8	42.4	0.8
mol002773	beta-carotene	537	37.2	0.6
mol000354	isorhamnetin	316.3	49.6	0.3
mol000358	beta-sitosterol	414.8	36.9	0.8
mol000359	sitosterol	414.8	36.9	0.8
mol000422	kaempferol	286.3	41.9	0.2
mol000433	FA	441.5	69	0.7
mol000449	stigmasterol	412.8	43.8	0.8
mol000492	(+)-catechin	290.3	54.8	0.2
mol005100	5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)chroman-4-one	302.3	47.7	0.3
mol006756	schottenol	414.8	37.4	0.8
mol000073	ent-epicatechin	290.3	49	0.2
mol000953	CLR	386.7	37.9	0.7
mol000098	quercetin	302.3	46.4	0.3

Candidate targets

By means of the four available resources, namely, the Gene cards, OMIM, Drug Bank and PharmGkb databases, 394 target genes were obtained. After merging HAS-related targets and active compound targets by venny website, 48 overlapping targets were recognized as candidate targets (see Figure 1).

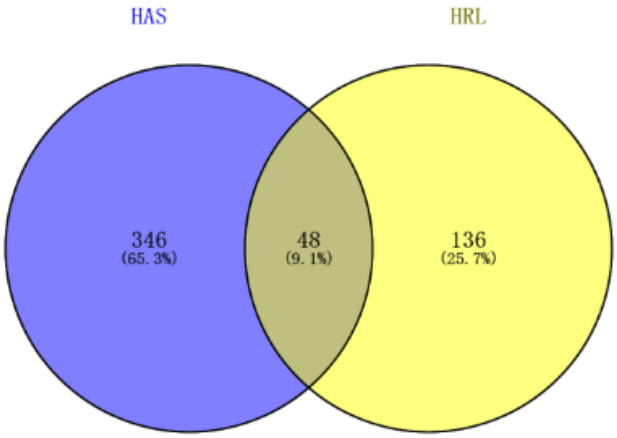

Figure1: The overlapping target after merging HAS-related targets and active compound targets.

PPI network analysis

Based on 48 candidate targets, PPI network was constructed by importing the gene ID of the candidate targets to the String database. Next, cytoscape 3.7.2 was used to visualize the PPI network. Based on the results of topological property analysis of the PPI, targets above the median were selected as the core targets (AKT1, MAPK14, CAT, TP53, AR, HIF-1A, VEGFA, NR3C1, CXCL8), and visualized by CytoNCA plug-in (see Figure 2).

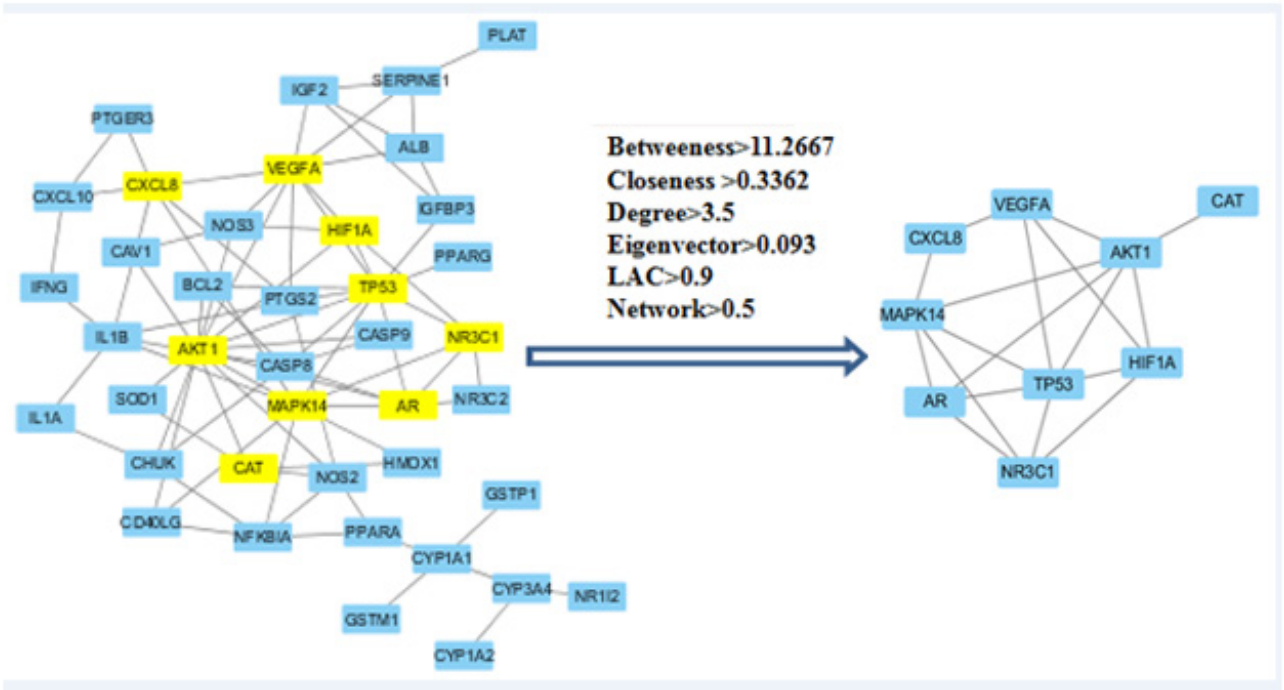

Figure 2: Hub genes of identified targets.

GO enrichment analysis

To reveal the pharmacological mechanisms of HRL in HAS, 48 overlapping targets were put into the Metascape database for annotation GO enrichment analysis including biological process, molecular function and cellular component. The top 10 GO analysis results were screened, with $P < 0.01$ serving as the threshold, as shown in Figure 3. Among these terms, the biological processes were mostly related to the response to lipopolysaccharide, response to nutrient levels, reactive oxygen species metabolic process, monocarboxylic acid metabolic process, response to peptide, response to oxygen levels, positive regulation of cell migration, cellular response to organic cyclic compound, extrinsic apoptotic signaling pathway, regulation of reactive oxygen species metabolic process. These results help to elucidate the biological function changes in the body after treatment with HRL.

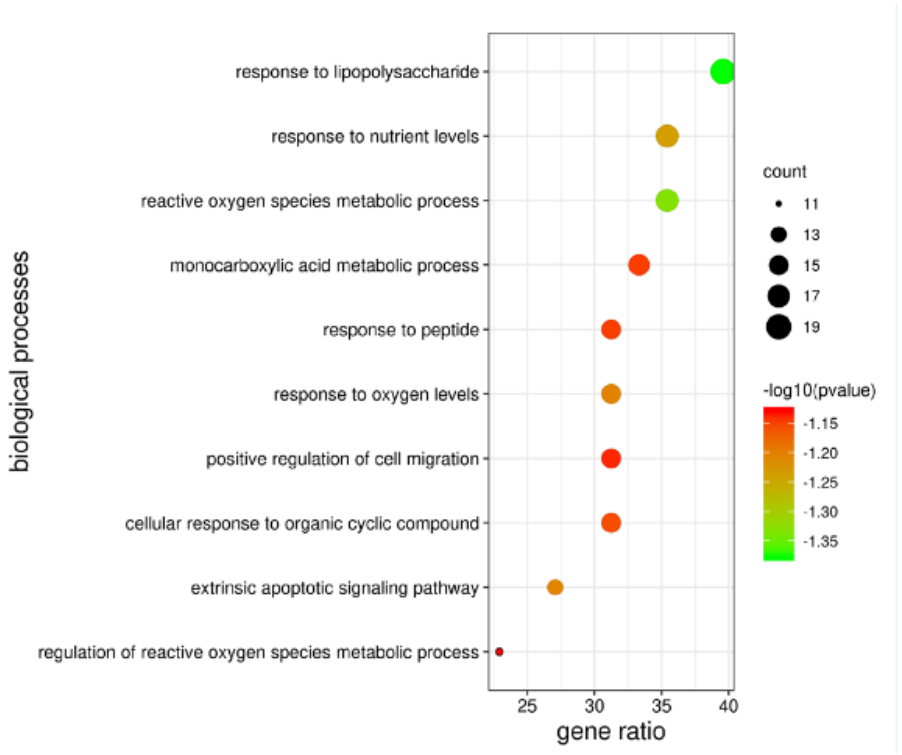

Figure 3: Gene Ontology analysis of potential targets of HRL.

KEGG pathway enrichment analysis

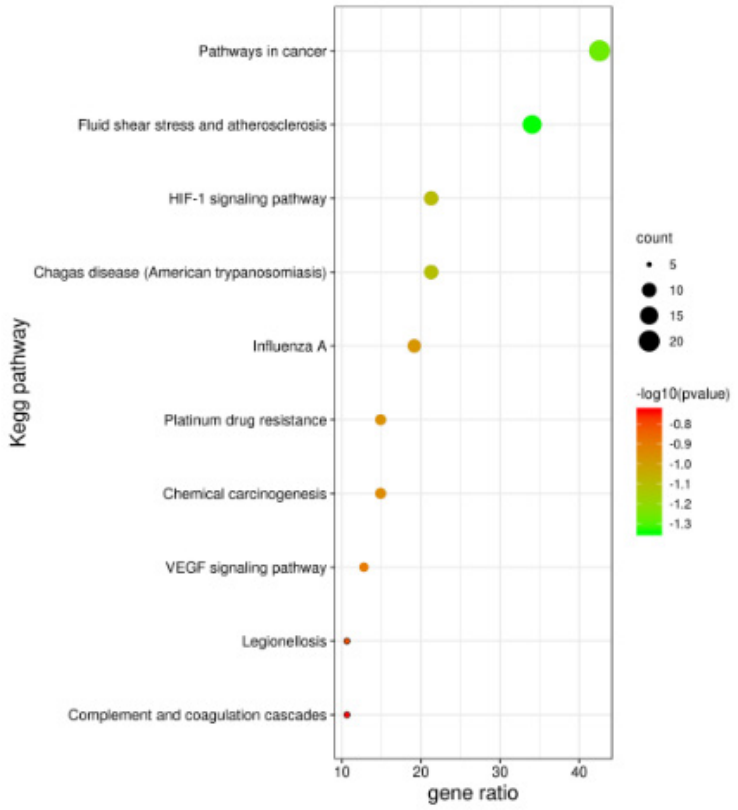

Figure 4: KEGG enrichment analysis of potential targets of HRL in treating HAS.

KEGG pathway enrichment analysis of the 48 candidate targets was obtained through the Metascape database. Based on the "count" values and P ≤ 0.05, a total of 16 pathways were obtained, and the top 10 pathways are shown as the core pathways in Figure 4. The results indicate that the mechanisms of HRL against HAS were related to pathways in cancer (hsa05200), fluid shear stress and atherosclerosis (hsa05418), HIF-1 signaling pathway (hsa04066), VEGF signaling pathway (hsa04370), complement and coagulation cascades, and so forth.

Compound-target network analysis

The former results showed that 16 compounds and 48 targets may be the bio-active substances and the pharmaceutical targets for HRL in the treatment of AMS. Based on the compounds and predicted targets, we constructed a network using Cytoscape 3.7.2. As presented in Figure 5, the yellow nodes represent active compounds, and the blue nodes represent potential targets. The edges represent the interaction between them, and the node sizes are proportional to the node degrees. The network indicated the potential relationships between the compounds and the targets, thereby revealing the potential pharmacological mechanisms of HRL for the treatment of HAS. Through topological analysis, we selected the compounds with the highest degree value as the core compounds. As shown in Figure 5, quercetin, kaempferol and pelargonidin, are the core compounds in HRL (degree=40), (degree=16) and (degree=8), respectively.

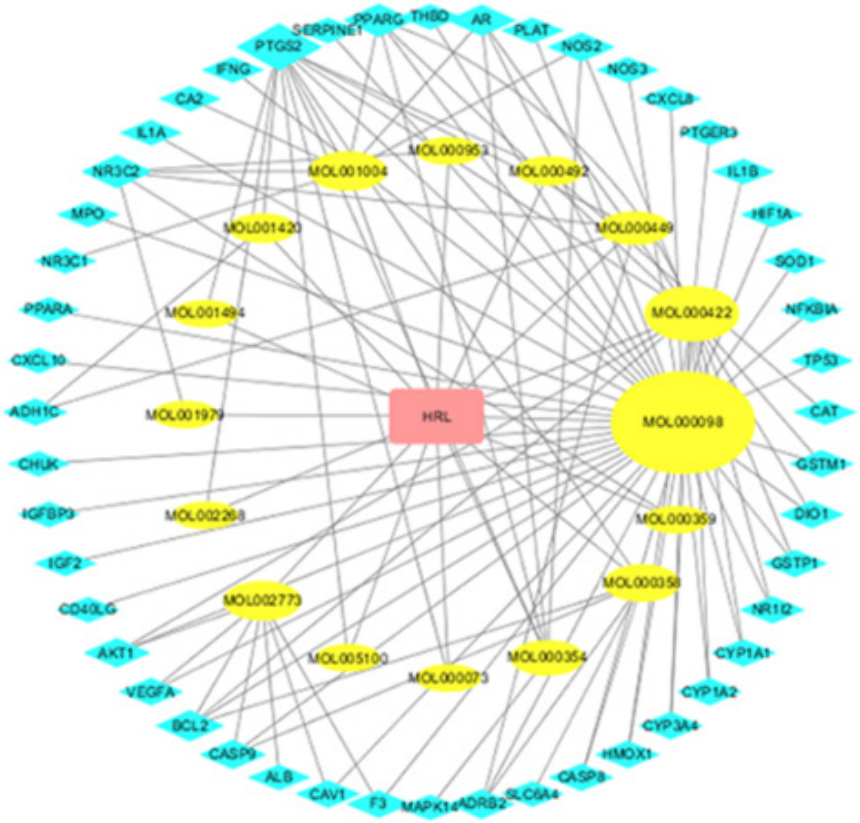

Figure 5: HRL-HAS disease network. Yellow circles represent active compounds of HRL, blue diamonds are potential targets of HRL for the treatment of HAS.

Docking results analysis

Based on the PPI network, we selected 9 core target genes for molecular docking. Affinity (kcal/mol) was used to value the score for the molecular docking, and an affinity < −7 indicated a stronger binding activity [14]. In Table 2, the affinity scores (< −7) of the former three ingredients obtained in 3.6 and targets are shown, which meant the components could act on the targets. The results indicated that the molecular docking results were consistent with the PPI screening results, and the reliability of hub gene was verified by molecular docking. The docking results of HRL to the HAS targets were shown in Table 2, and Figure 6 was presented to show the combination of quercetin and six targets including AKT1, AR, CXCL8, HIF-1A, VEGFA and TP53 in a 3D graph.

Table 2: The affinitlies (kcal/mol) of ingredients and relative targets.

Ingredient	Targets	Affinitlies (kcal/mol)
Pelargonidin	AR	-8.7
Kaempferol	AR	-9.3

Quercetin	AR	-8.6
Kaempferol	AKT1	-9
(+) Catechin	CAT	-9.8
Quercetin	CXCL8	-7.1
Quercetin	HIF1A	-8.5
Isorhamnetin	MAPK14	-8.5
Pelargonidin	NR3C1	-9.1
Quercetin	TP53	-7.2
Quercetin	VEGFA	-7.5
Quercetin	AKT1	-10.5

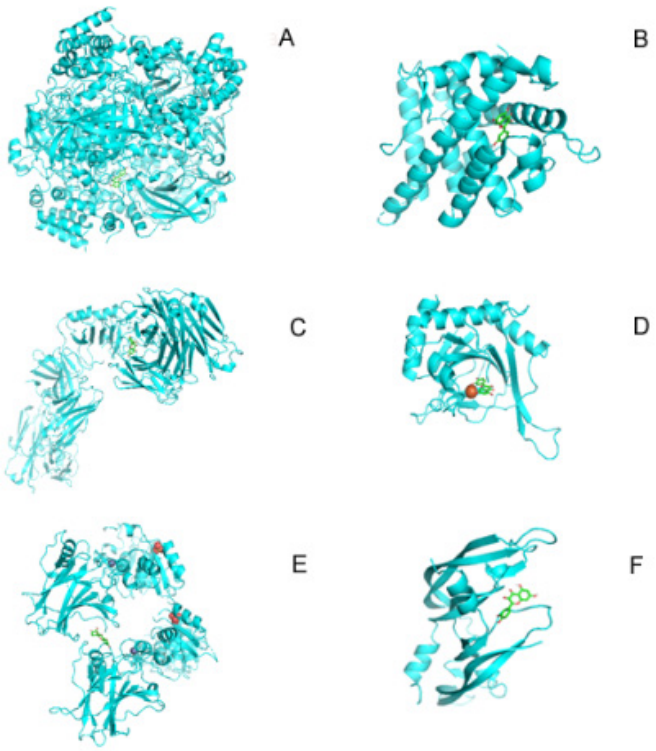

Figure 6: Molecular docking of quercetin with different targets.
(a) Docking process of quercetin with AKT1, (b) Docking process of quercetin with AR.
(c) Docking process of quercetin with CXCL8, (d) Docking process of quercetin with HIF-1A.
(e) Docking process of quercetin with TP53, (f) Docking process of quercetin with VEGFA.

Conclusion

In this study, there were 33 active ingredients (16 key active ingredients) in HRL, and 48 targets were screened out for HAS treatment. Through enrichment analysis of GO biological processes and KEGG signaling pathways, the therapeutic mechanisms of HRL on HAS may primarily involve the following effects: response to lipopolysaccharide, peptide, response to nutrient levels, regulate reactive oxygen species metabolic process, act on HIF-1 signaling pathway and VEGF signaling pathway, and extrinsic apoptotic signaling pathway. Moreover, it was preliminarily predicted that HRL may regulate the core targets (AKT1, MAPK14, CAT, TP53, AR, HIF-1A, VEGFA, NR3C1, and CXCL8) through quercetin, kaempferol and pelargonidin, which have been validated by molecular docking. To date, some of the predicted targets such as HIF-1A, VEGFA, NR3C1 have been confirmed by published literatures [15-19], moreover, Dexamethasone, which acts on NR3C1 has been used in clinical practice of HAS treatment [20, 21]. As for AR (androgen receptor), which several components including pelargonidin, quercetin, and kaempferol could bind tightly to it (Table. 2) and many researchers have reported the correlation between HAS and sex [22, 23], however, the internal mechanism of HRL acting on AR needed further research. This study not only explored the potential molecular mechanisms regarding the anti-hypoxia effect of active components in HRL, which laid the foundation for further investigation, but also presented novel clues for the development and utilization of HRL resources.

Statement of Ethics

Not applicable.

Acknowledgement

None.

Conflict of Interest

No conflict of interest.

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