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Mechanism of Olibanum and Myrrha for the Acute Soft Tissue Injury Based on Network Pharmacology

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Abstract

Objective The objective of this study was to screen the therapeutic target of olibanum and myrrha on acute soft tissue injury (ASTI) by network pharmacology and to clarify their mechanisms.

Methods The main chemical constituents and the targets of olibanum and myrrha were obtained by using traditional Chinese medicine systems pharmacology database and analysis platform database. The disease targets of ASTI were searched by GeneCards. The intersection targets of herbs and diseases were selected for protein interaction analysis, protein–protein interaction network was constructed, and potential protein functional modules in the network were explored. A compound–target–disease network was constructed using Cytoscape3.8.2 software. The targets were analyzed by gene ontology analysis and Kyoto Encyclopedia of Genes and Genomes enrichment analysis based on the Metascape database.

Results The core active components of olibanum and myrrha were quercetin, β-sitosterol, and stigmasterol. The core targets were PGR, NCOA2, PTGS2, PRKCA, and NR3C2. Pathways in cancer, AGE-RAGE signaling pathway in diabetic complications might play a potential role in olibanum and myrrha in the treatment of ASTI.

Conclusion Olibanum and myrrha have the characteristics of multiple components, multiple targets, and overall regulation in the treatment of ASTI.

Keywords
- olibanum and myrrha
- acute soft tissue injury
- network pharmacology

Introduction

Acute soft tissue injury (ASTI) is a kind of trauma syndrome caused by direct or indirect violent injury of various malformations, which is a common orthopedic disease.[1] It is mostly manifested as local edema, muscle fiber rupture, pain, and dysfunction of soft tissues (ligaments, fascia, tendons, synovium, muscles, fat, nerves and blood vessels around the joint capsule, etc.).[2] ASTI belongs to the category of "acute tendon injury" in traditional Chinese medicine (TCM). It is caused by external forces damaging qi and blood from outside and inside, qi stagnation and blood stasis, and obstructing the meridians, resulting in pain, swelling, local blue and purple, and activity disorder.[3] Nowadays, with the continuous development of medicine, there are many methods in TCM and Western medicine to treat ASTI. The conservative treatment of western medicine and the application of new technology complement each other. TCM treatment of ASTI is based on the basic treatment principles of orthopaedic and traumatology, including equal emphasis on muscles and bones, internal and external treatment, and phased use of medicine, forming the multi-method and reference treatment methods

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of TCM, such as internal and external treatment, acupuncture, and manipulation.[4]

Olibanum and myrrha are compatible with TCM commonly used in the treatment of ASTI. Olibanum and myrrha belong to herbs for promoting blood circulation and removing blood stasis. Olibanum is spicy, bitter, and warm, promoting blood circulation and calming pain, reducing swelling and developing muscle. Myrrha is good at removing blood stasis and regulating blood, and the two must be used together to promote the functions of zang-fu organs, meridians, and collaterals.[5] Network pharmacology is a multidisciplinary discipline that explains the relationship between herbs and diseases from a molecular perspective as well as a systematic and holistic perspective and shows the systematic pharmacological mechanism of herbs.[6] Network pharmacology studies herbs from the perspective of "multi-component, multi-target and multi-approach," which is consistent with the holistic concept of TCM and the treatment concept of syndrome differentiation.[7] Many studies have found that olibanum and myrrha have significant efficacy in the treatment of ASTI. This paper used network pharmacology to identify the main active components and core targets of olibanum and myrrha in the treatment of ASTI and further analyze the molecular mechanism of its treatment of ASTI.

Methods

Olibanum and Myrrha Search for Active Ingredients

Using the traditional Chinese medicine systems pharmacology database and analysis platform (TCMSP) database (http://tcmspw.com/tcmsp.php), we searched for "olibanum" and "Myrrha" fully effective components.

Screening of Active Ingredients and Collection of Potential Targets

Oral availability $\geq$30% and drug-like property $\geq$0.18 were used to screen the active ingredients and their protein targets. Protein targets were then standardized in the Uniprot Protein Database (https://www.uniprot.org/).

Identification of Targets Associated with Acute Soft Tissue Injury

Keywords related to ASTI, "acute soft tissue injury," "acute closed soft tissue injury," "acute non-open soft tissue injury," etc., were used to collect disease-related targets from GeneCards database (http://www.genecards.org/) and OMIM database (https://omim.org/). ASTI targets were obtained by combining and deleting duplicate values.

Protein–Protein Interaction Network Construction

The intersection of olibanum and myrrha targets with targets related to ASTI was plotted, and a Venn diagram was drawn. Intersection targets were submitted to String11.5 database (https://www.string-db.org/) and protein–protein interaction (PPI) network was constructed. "Homo sapiens" was selected as biological species, the minimum interaction score was set as " $>$ 0.9," the nodes of network disconnection were hidden, and the other settings were kept as default settings to obtain the PPI network. The potential core targets can be obtained by the degree of connectivity.

Gene Ontology Pathway Enrichment Analysis and KEGG Pathway Enrichment Analysis

To explore the role of target proteins of olibanum and myrrha in gene function and find the core pathway of olibanum and myrrha in ASTI, this study used Metascape database (https://metascape.org/) to conduct enrichment analysis of gene ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway for core targets. Microscopic letter cloud platform (http://www.bioinformatics.com.cn/) was used for visualization processing results.

Construction of Composition–Target–Disease Network Diagrams

The active ingredients and screened core targets were uploaded to Cytoscape 3.8.2 software to generate "component–target–disease network map." The built-in tool CytoScape3.8.2 was used to analyze network topological parameters of active components and targets and identify core targets and main active components according to relevant parameters.

Molecular Docking Verification

PDB database (https://www.rcsb.org/pages/search_features) was used to download the key targets of PDB structure, downloading and removing stasis in TCMSP database gave birth to a new capsule MOL2 structure of active ingredients. AutoDock Vina 1.1.2 was used to dock the molecule and target. If the binding energy was less than 0, there was binding activity between ligand and receptor. If the binding energy was less than $-5\,\mathrm{kcal{\cdot}mol^{-1}}$, the docking was good. Finally, Pymol 2.2.0 was used to visualize the results.

Results

Acquisition of Active Components and Targets of Olibanum and Myrrha

A total of 403 active ingredients of olibanum and myrrha were collected by TCMSP, including 127 olibanum and 276 myrrh. A total of 36 active ingredients of olibanum and myrrh were obtained by ADME screening, including 5 olibanum and 31 myrrh, as shown in ►**Table 1**. There were 11 targets for olibanum and 175 targets for myrrh. A total of 186 targets for olibanum and myrrh were obtained after combination.

Acquisition of Targets Associated with Acute Soft Tissue Injury

The search results of disease-related targets of ASTI were as follows: 4,448 from GeneCards database and 207 from OMIM database, excluding repeated targets, a total of 4,600 ASTI-related targets.

Results of Intersection Target Screening and Protein–Protein Interaction Network Construction

The intersection of the active ingredient targets of screened olibanum and myrrha and the targets of ASTI diseases was

Table 1 Olibanum and myrrha information on active ingredients

Source	MOLID	Effective constituent	OB/%	DL
Olibanum	MOL001215	Tirucallol	42.12	0.75
Olibanum	MOL001241	O-acetyl-α-boswellic acid	42.73	0.7
Olibanum	MOL001243	3α-Hydroxy-olean-12-en-24-oic-acid	39.32	0.75
Olibanum	MOL001255	Boswellic acid	39.55	0.75
Olibanum	MOL001295	Phyllocladene	33.4	0.27
Myrrha	MOL001001	Quercetin-3-O-β-D-glucuronide	30.66	0.74
Myrrha	MOL001002	Ellagic acid	43.06	0.43
Myrrha	MOL001004	Pelargonidin	37.99	0.21
Myrrha	MOL001006	Poriferasta-7,22E-dien-3β-ol	42.98	0.76
Myrrha	MOL001009	Guggulsterol-VI	54.72	0.43
Myrrha	MOL001013	Mansumbinoic acid	48.1	0.32
Myrrha	MOL001026	Myrrhanol C	39.96	0.58
Myrrha	MOL001028	(8R)-3-Oxo-8-hydroxy-polypoda -13E,17E,21-triene	44.83	0.59
Myrrha	MOL001029	Myrrhanones B	34.39	0.67
Myrrha	MOL001031	Epimansumbinol	61.81	0.4
Myrrha	MOL001033	Diayangambin	63.84	0.81
Myrrha	MOL001040	(2R)-5,7-Dihydroxy-2-(4-hydroxyphenyl) chroman-4-one	42.36	0.21
Myrrha	MOL001045	(13E,17E,21E)-8-Hydroxypolypodo-13,17,21-trien-3-one	44.34	0.58
Myrrha	MOL001046	(13E,17E,21E)-Polypodo-13,17,21-triene-3,18-diol	39.96	0.58
Myrrha	MOL001049	16-Hydroperoxymansumbin-13(17)-en-3β-ol	41.05	0.49
Myrrha	MOL001052	Mansumbin-13(17)-en- 3,16-dione	41.78	0.45
Myrrha	MOL001061	(16S, 20R)-Dihydroxydammar-24-en-3-one	37.34	0.78
Myrrha	MOL001062	15α-Hydroxymansumbinone	37.51	0.44
Myrrha	MOL001063	28-Acetoxy-15α-hydroxymansumbinone	41.85	0.67
Myrrha	MOL001095	Isofouquierone	40.95	0.78
Myrrha	MOL001126	[(5aS,8aR,9R)-8-oxo-9-(3,4,5-trimethoxy-phenyl)-5,5a,6,9-tetrahydroisobenzofur-ano[6,5-f][1,3]benzodioxol-8a-yl] acetate	44.08	0.9
Myrrha	MOL001131	phellamurin_qt	56.6	0.39
Myrrha	MOL001138	(3R,20S)-3,20-Dihydroxydammar- 24-ene	37.49	0.75
Myrrha	MOL001156	3-Methoxyfuranoguaia-9- en-8-one	35.15	0.18
Myrrha	MOL001175	Guggulsterone	42.45	0.44
Myrrha	MOL000358	β-sitosterol	36.91	0.75
Myrrha	MOL000449	Stigmasterol	43.83	0.76
Myrrha	MOL000490	Petunidin	30.05	0.31
Myrrha	MOL000098	Quercetin	46.43	0.28
Myrrha	MOL000988	4,17(20)-(cis)-pregnadiene-3,16-dione	51.42	0.48
Myrrha	MOL000996	Guggulsterol IV	33.59	0.74

Abbreviations: DL, drug-like property; OB, oral availability.

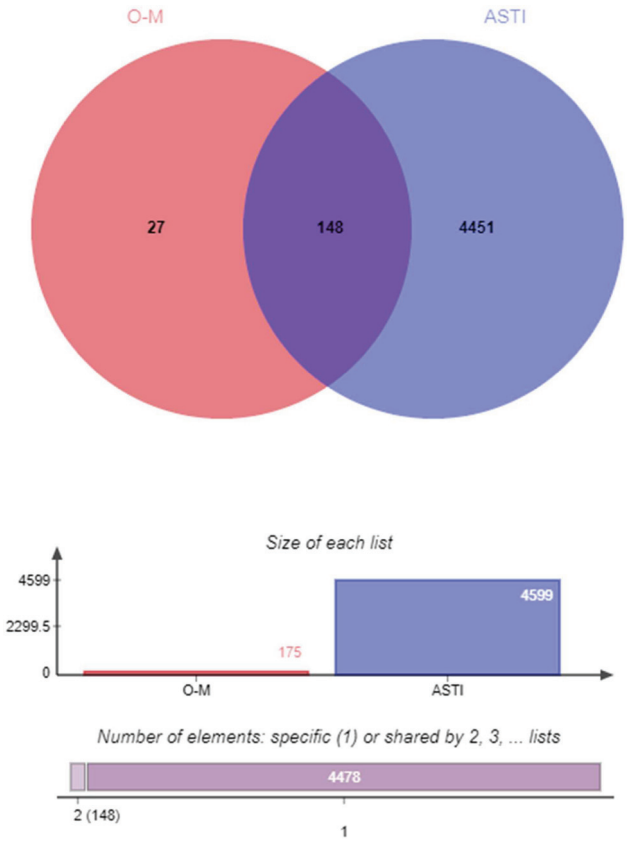

Fig. 1 *Olibanum and myrrha targets at the intersection of* ASTI. Notes: O–M represents olibanum and myrrha; ASTI represents acute soft tissue injury.

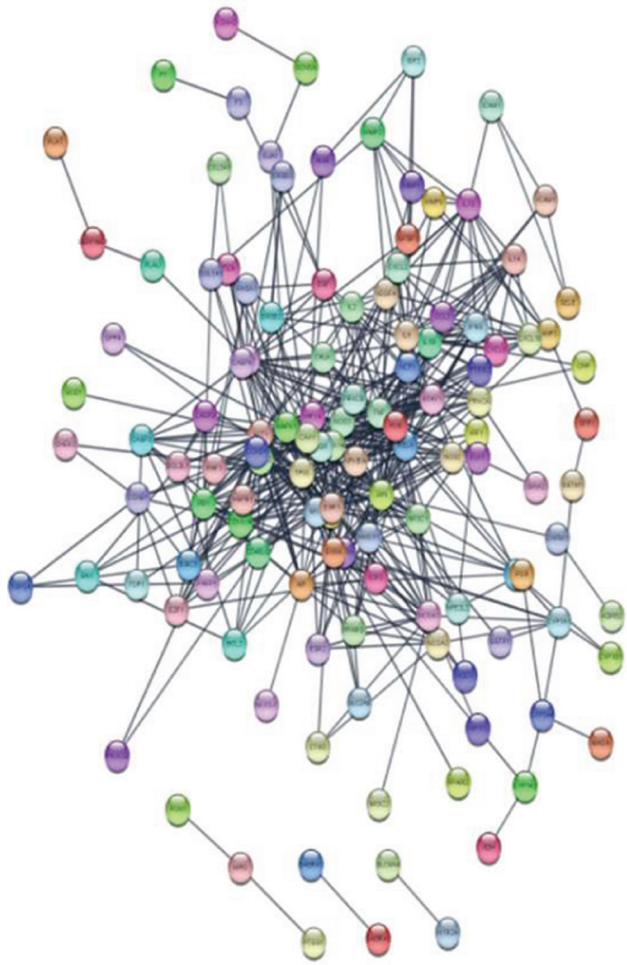

Fig. 2 PPI network diagram.

obtained by drawing Venn diagram,[8] and 148 common targets of olibanum and myrrha on the active ingredient, ASTI, were obtained, as shown in ►**Fig. 1**. Then, the intersection targets were submitted to STRING11.5 platform, and the PPI network diagram of olibanum and myrrha targets was obtained, as shown in ►**Fig. 2**. PPI Network map had 158 nodes with 585 edges, and the average node degree was 7.41. According to the connection degree, the core genes of PPI network were JUN, TP53, AKT1, MAPK1, RELA, etc.

Gene Ontology Pathway Enrichment and KEGG Pathway Enrichment Analysis Results

Metascape gene annotation and analysis resource platform were used to conduct GO pathway enrichment analysis and KEGG pathway enrichment analysis for 148 targets, and micro-bioinformatics cloud platform was used to visually process the enrichment analysis results, as shown in ►**Fig. 3**. GO pathway enrichment analysis set the basic screening conditions for biological process, cell component, and molecular function enrichment analysis, and screened the top 20 enrichment analysis results in each group. The results showed that the function of multiple targets was closely related to the treatment of ASTI. The main biological processes involved in olibanum and myrrha include reaction to exogenous stimulation, cell reaction to organic ring compounds, reaction to injury, reaction to lipolysaccharides, etc. The results of cell component analysis mainly included membrane raft, plasma membrane protein complex, dendrite, transcription regulatory complex, etc. The results of molecular function analysis mainly included transcription factor binding, nuclear receptor activity, protein domain-specific binding, protein kinase binding, and so on. A total of 334 pathways were obtained from KEGG pathway enrichment analysis, indicating that the main pathways of olibanum and myrrha in the treatment of ASTI include cancer pathway and AGE-RAGE signaling pathway in diabetic complications, as shown in ►**Fig. 4**.

Construction of Component–Target–Pathway Network Diagram

Thirty-six active components of olibanum and myrrha and 148 common targets of olibanum and myrrha and ASTI were uploaded to Cytoscape3.8.2 software to construct a "component-target-pathway network diagram," as shown in ►**Fig. 5**. NetworkAnalyzer (built-in tool of CytoScape3.8.2) was used to analyze the network topology parameters of olibanum and myrrha for the treatment of ASTI, and the core components and core targets were obtained. The results showed that the connection degree of quercetin, the main component of

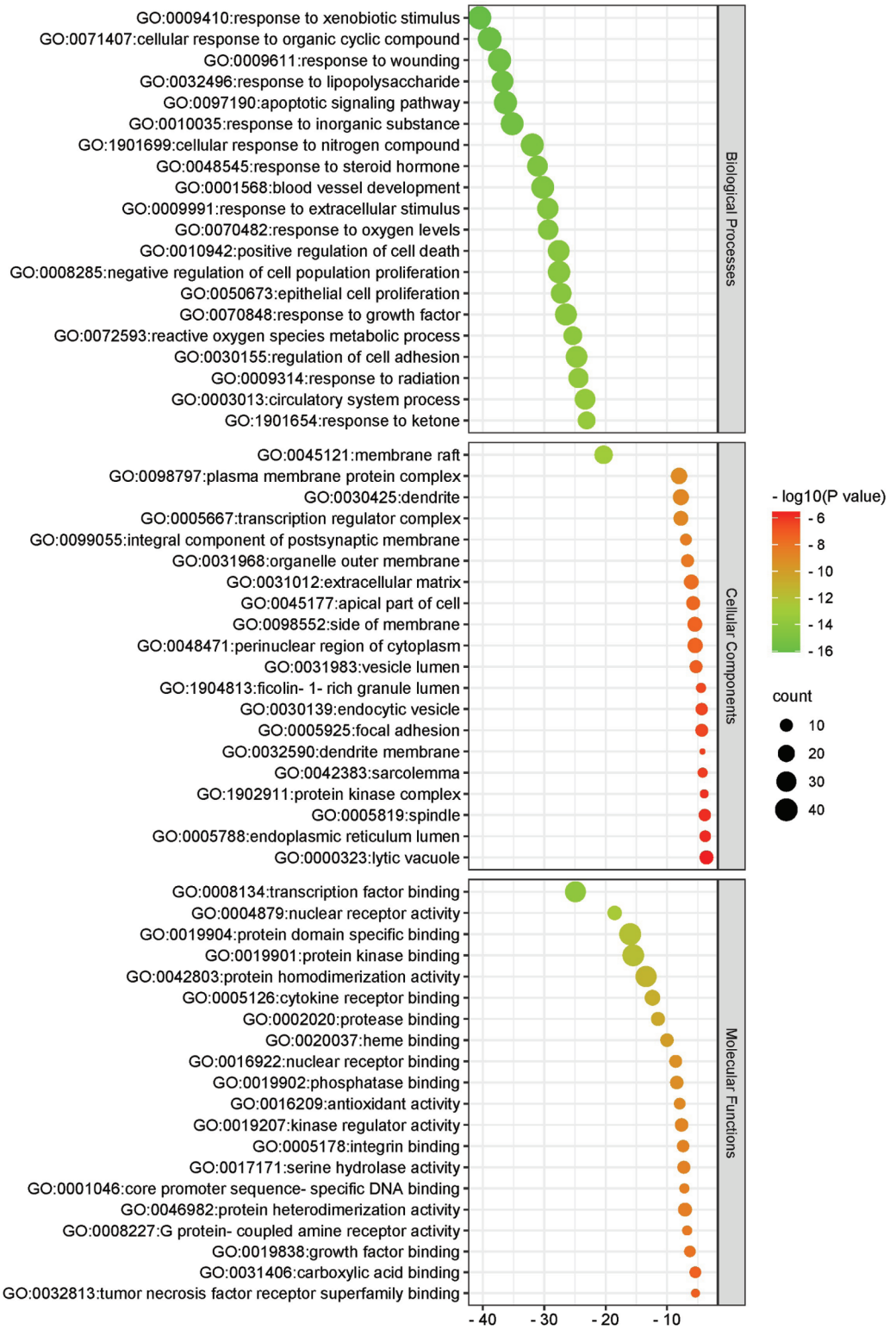

Fig. 3 GO pathway enrichment analysis.

olibanum and myrrha, in the treatment of ASTI was 123%, the middle degree was 0.5789 and the density was 0.6078. The connection degree of β -sitosterol was 26, the intermediate degree was 0.0753, and the compact degree was 0.3949. The degree of connection, medium, and compactness of stigmasterol were 21, 0.0756, and 0.3816, respectively. β -sitosterol and stigmasterol were also predicted to play an important role in the treatment of ASTI with olibanum and myrrha, as shown in ►**Table 2**. Among the target analysis results, PGR ranked first in terms of connectivity 19, mediality 0.051, and tightness 0.3406. It was predicted that PGR was the core target of olibanum and myrrha for the treatment of ASTI.

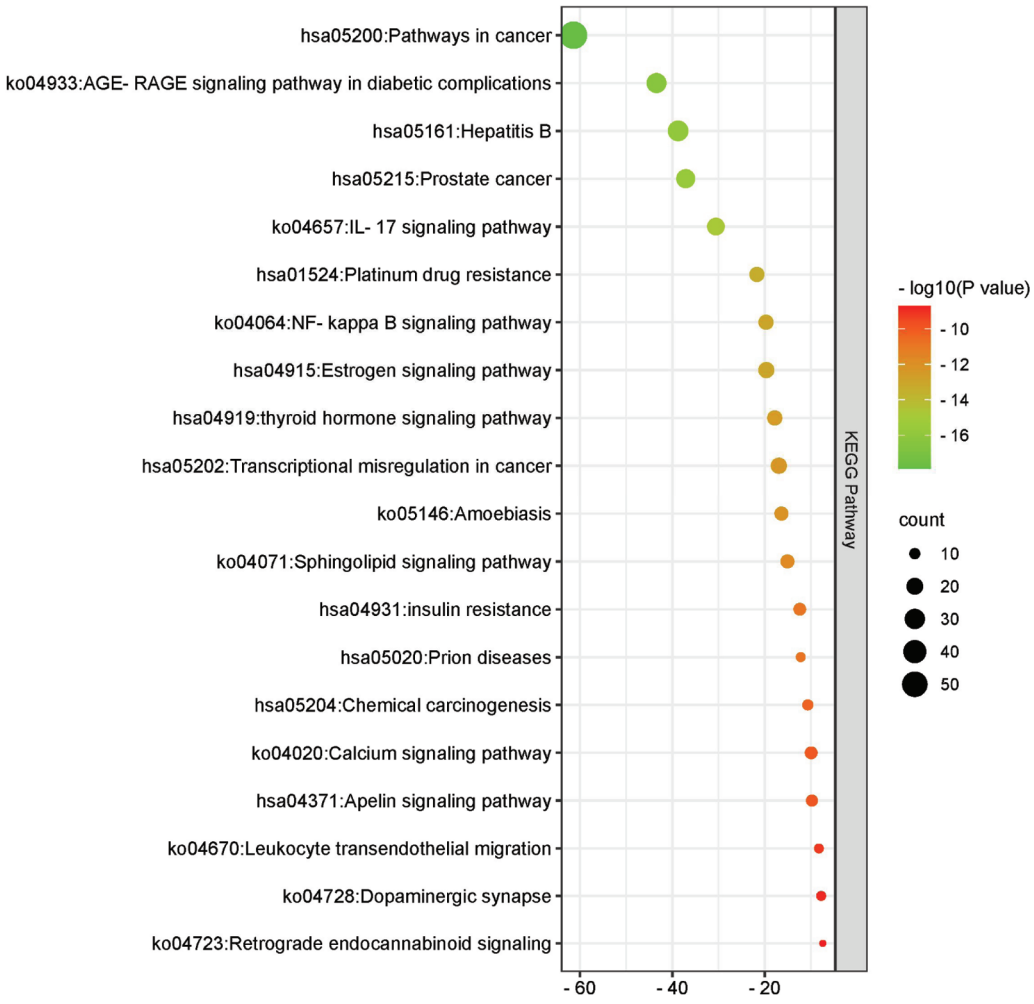

Fig. 4 KEGG pathway enrichment analysis.

NCOA2, PTGS2, PRKCA, NR3C2, PRKCB, MAPK1, AKT1, RXRA, RELA also played an important role in the treatment of olibanum and myrrha in ASTI, as shown in ►**Table 3**.

Molecular Docking Verification Results

The active components of olibanum and myrrha obtained by analysis were quercetin and β-sitosterol, and the core targets of molecular docking were PGR, NCOA2, and PTGS2. The results showed that the binding energy between each active ingredient and the core target was less than $-5 kcal \cdot mol^{-1}$, and all had strong binding activity, as shown in ►**Table 4**. Finally, the molecular docking results were visualized, as shown in ►**Fig. 6**.

Discussion

ASTI is the soft tissue injury of motor system caused by many acute injury factors. Its therapeutic principles have been developed over the years through observation and experimentation. In the principle of ICE (ice, compression, and elevation of the injured site), ICE can relieve pain in the acute stage of ASTI. Studies have found that ICE can improve pain threshold and pain tolerance and significantly reduce nerve transmission speed.[9] Compression bandaging reduces swelling, limits the amount of edema caused by fluid leaking into the tissue from damaged capillaries, controls inflammatory exudation and reduces fibrin, thereby reducing scar tissue production, and helps control the osmotic pressure of tissue fluid in the injured area. Elevation of the injured site reduces pressure on local blood vessels, increases the drainage of inflammatory exudate through lymphatic vessels, and reduces and limits edema and its associated complications. RICE principles (rest, ice, compression, and elevation) add "Rest" to the ICE principle. Rest reduces the metabolic demands of the injured tissue, thereby avoiding an increase in blood flow. Rest avoids increased stress on injured tissue and reduces the breakdown of fibrin bonds, the first element of the repair process.[10] PRICE principle (protection, rest, ice, compression, and elevation) can reduce secondary injury. "Protection" principle is added to RICE principle. PRICE principle carries out the concept of rehabilitation of ASTI in quiescent treatment to the end. Studies have found that PRICE principle can reduce microvascular blood flow and down-regulate intramural tissue perfusion after injury.[11] With the vigorous development of modern sports rehabilitation technology, the latest concepts such as early

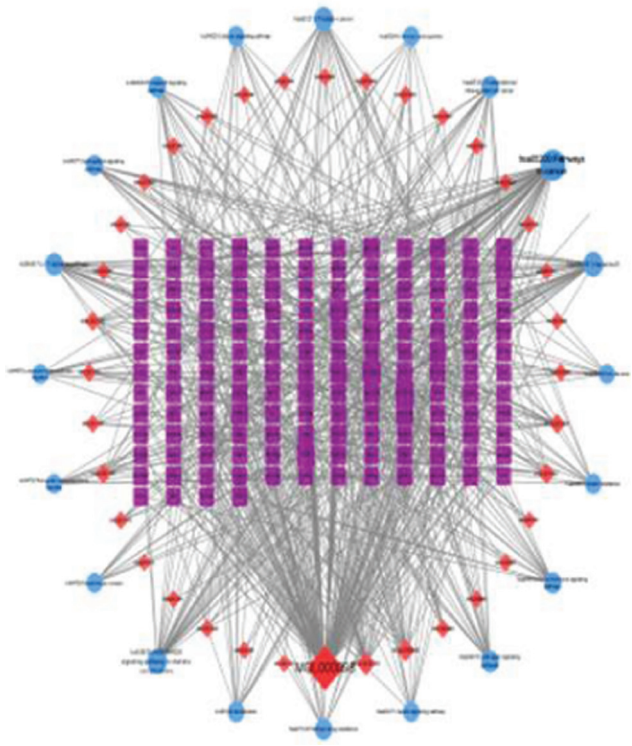

Fig. 5 *Component–target–pathway network diagram.* Notes: circle is the pathway; diamond is the active component; rectangle is the target; the larger the node area and darker the color, the more important the node is.

Table 4 Molecular docking of core components and targets of Quyushengxin capsule

Component	PGR	NCOA2	PTGS2
	kcal/mol^{-1}	kcal/mol^{-1}	kcal/mol^{-1}
Quercetin	−9.1	−6.8	−9.7
Beta-sitosterol	−11.5	−7.5	−9.1

rehabilitation therapy and personalized rehabilitation formulation have also emerged[12]. Functional therapy and progressive mechanical loading therapy have emerged, followed by "optimal loading" to replace the rest principle, resulting in the emergence of POLICE principle.[13] With the continuous development and change of doctor–patient relationship and the increasingly clear doctor–patient cooperation, the PEACE & LOVE principle (PEACE: protection, elevation, avoid anti-inflammatory modalities, compression, educate; LOVE: load, optimism, vascularization, exercise) highlights the importance of educating patients and dealing with social and psychological factors to promote recovery and for the first time proposes that the premature use of anti-inflammatory drugs should be avoided to achieve the purpose of inflammation repair.[14]

With the development of society, ASTI has become an increasingly frequent accidental injury in people's fast-paced life and work, and its influence on people's work and life is increasing year by year. With the continuous progress of

Table 2 Characteristic parameters of main active ingredient network nodes of olibanum and myrrha

MOLID	Name	Degree	Betweenness centrality	Closeness centrality
MOL000098	quercetin	123	0.578911968	0.607784431
MOL000358	β-sitosterol	26	0.075295448	0.394941634
MOL000449	Stigmasterol	21	0.075554792	0.381578947
MOL001002	ellagic acid	15	0.015257533	0.365107914
MOL001004	pelargonidin	13	0.030286544	0.367753623
MOL001156	3-methoxyfuranoguaia-9- en-8-one	11	0.011790874	0.354895105
MOL001126	[(5aS,8aR,9R)-8-oxo-9-(3,4,5-trimethoxyphenyl)-5,5a,6,9-tetrahydroisobenzofurano[6,5-f][1,3] benzodioxol-8a-yl] acetate	11	0.016820228	0.340604027
MOL000490	petunidin	7	0.004221262	0.351211073
MOL001138	(3R,20S)-3,20-dihydroxydammar- 24-ene	6	0.003297119	0.333881579
MOL001131	phellamurin_qt	6	0.007189137	0.346416382

Table 3 Node characteristic parameters of core target network of olibanum and myrrha

The core target	Degree	Betweenness centrality	Closeness centrality	The core target	Degree	Betweenness centrality	Closeness centrality
PGR	19	0.050982856	0.340604027	PRKCB	14	0.017169956	0.435622318
NCOA2	19	0.071779148	0.472093023	MAPK1	13	0.010768025	0.422916667
PTGS2	19	0.072324782	0.476525822	AKT1	12	0.010603844	0.422916667
PRKCA	15	0.024305536	0.445175439	RXRA	12	0.042261508	0.453125
NR3C2	15	0.027280353	0.295918367	RELA	12	0.006806169	0.415983607

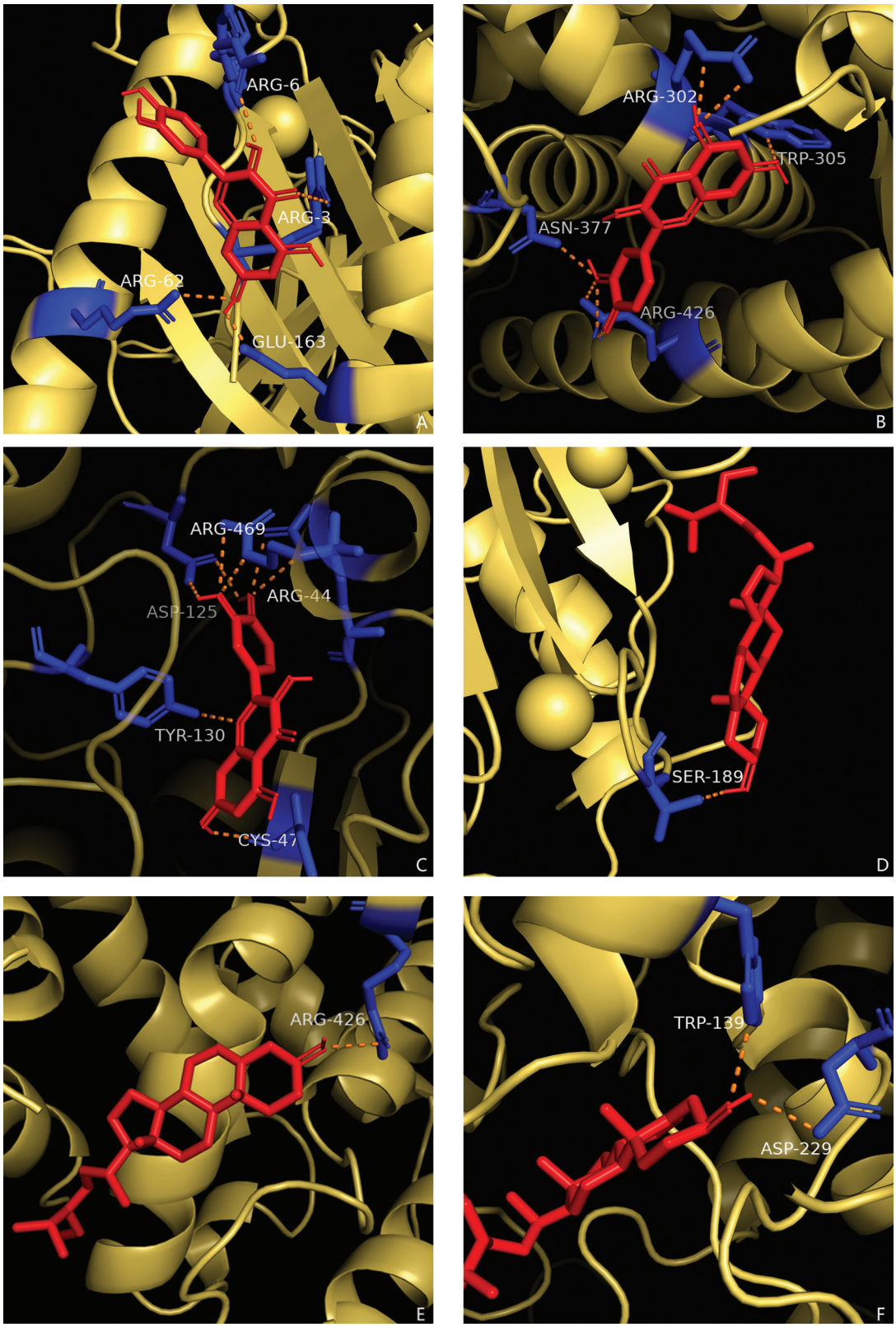

Fig. 6 Molecular docking verification.

medical technology and people's increasing attention to their own health, there are more and more methods of treating ASTI with Chinese and Western medicine. Internal and external treatment of TCM, modern Chinese medicine for external use, oral medicine of Western medicine, physical therapy, regenerative medicine, etc., all achieve local detumescence, analgesic, anti-inflammatory, and even systemic recuperation of ASTI under the action of various principles. Jiang et al[15] established the ASTI model of hammer-hit male SD rats, and the experiment showed that Qingre Huoxue

Jiedu decoction could treat ASTI by reducing the levels of inflammatory factors such as TNF-α, IL-6, PGE2, and IL-1β in muscle tissue, regulating Mir-26B-5P/COX2 axis and inhibiting the inflammatory response. Olibanum and myrrha are the main components of XuanHuang ointment, which have a significant curative effect on various bone injury diseases, including ASTI.[16,17] Experiments have proved that Xuanhuang ointment can significantly reduce the levels of IL-6, IL-1β, TNF-α, and PGE2 in animal injured tissues and also reduce hemorheological indicators. It is important to enhance and improve the immune function of experimental animals, protect the immune organs, so as to achieve the purpose of anti-inflammatory analgesia, increase the immunity of the body, and treat ASTI.[18–20]

Olibanum and myrrha have been used for a long time in the treatment of ASTI such as glinting, twisting, tumbling and injury, and are compatible with Qili Powder,[21] Jiufen Powder,[22] Huoluoxiao Ling Dan.[23] Using the method of network pharmacology, starting from the material basis of olibanum and myrrha, this study discusses the molecular mechanism of olibanum and myrrha in the treatment of ASTI, and provides a certain theoretical basis for the follow-up study of olibanum and myrrha in the treatment of ASTI. After analysis, the main active ingredient of olibanum and myrrha in the treatment of ASTI was predicted to be quercetin. Various studies have shown that quercetin has the functions of anti-oxidation, anti-cancer, hypoglycemic, anti-inflammatory, antihypertensive, antiviral, anti-oxidation, lipid regulation, cardiovascular protection, and bone protection.[24] PGR is a core target of olibanum and myrrha in the treatment of ASTI. The pathway prediction of olibanum and myrrha in the treatment of ASTI may be related to cancer pathway and AGE-RAGE signaling pathway in diabetic complications.

The results showed that the compound regulatory targets of olibanum and myrrha were complex, and the target intervention biological processes and signal pathways were diverse, which fully reflected the characteristics of olibanum and myrrh on multi-target and multi-pathway interaction. The results suggest the possibility and feasibility of regulating important targets in the network to regulate the entire network, which provides a scientific basis for the clinical application of olibanum and myrrha in the treatment of ASTI and also provides a new direction for exploring the potential mechanism of olibanum and myrrha. However, this paper only predicted the active components, target, and pathway information of olibanum and myrrha in the treatment of ASTI from the perspective of network pharmacology, without the support of relevant clinical trial research. Subsequent studies should also complete the related research content of olibanum and myrrha in the treatment of ASTI from the basic and clinical aspects.

Credit Authorship Contribution Statement

Miao Tan: Data collection and curation, formal analysis, software, and writing original draft. **Yan Cheng:** Conceptualization, methodology, supervision, and writing - review & editing.

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Conflict of Interest

The authors declare no conflict of interest.

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