Predicting Mechanism for *Myristica Fragrans* Houtt. in Treating Hepatocellular Carcinoma Through Network Pharmacology and Molecular Docking

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Abstract. Aim: This study aimed to evaluate the therapeutic potential and mechanisms of Myristica fragrans Houtt. (MF) in hepatocellular carcinoma (HCC) using network pharmacology and molecular docking. Methods: Active compounds of MF were identified via the TCMSP database, and corresponding targets were predicted using Systematic Pharmacology Database and Analysis Platform (TCMSP) and Swiss Target Prediction database. HCC-related targets were retrieved from GeneCards and intersected with MF targets to identify potential therapeutic targets. Protein-protein interaction (PPI) networks were constructed using STRING, and core targets were screened based on degree centrality. Functional enrichment analyses (GO and KEGG) were conducted via the DAVID database. Finally, molecular docking was performed to validate interactions between key compounds and core targets. Results: Nine active compounds of MF were identified, corresponding to 241 targets related to HCC. PPI analysis revealed 42 core targets, with AKT1, SRC, EGFR, HSP90AA1, and CASP3 as key nodes. Licarin B, threo-austrobailignan-5, and Kudos were identified as main active constituents. GO enrichment indicated involvement in biological processes such as apoptosis, protein phosphorylation, and cell proliferation. KEGG analysis highlighted key pathways including PI3K-AKT, VEGF, and hepatitis B signaling. Molecular docking confirmed strong binding affinities of Licarin B, threo-austrobailignan-5, and isoguaiacin to AKT1, SRC, and EGFR. Conclusion: Myristica fragrans exhibits therapeutic potential against HCC via multi-target, multi-component, and multi-pathway mechanisms. These findings provide a theoretical basis for further development of MF as a complementary treatment for HCC.

Keywords: *Myristica Fragrans* Houtt., Hepatocellular Carcinoma, Network Pharmacology, Molecular Docking, Signaling Pathway.

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1 Introduction

A severe hazard to human health is hepatocellular carcinoma (HCC), a malignant tumor with a high rate of morbidity and mortality [1]. The most effective method for hepatocellular carcinoma patients to attain long-term survival at the moment is surgical treatment, including hepatectomy and liver transplantation [2]. However, patients are frequently in the middle to late stages when hepatocellular carcinoma is diagnosed and are no longer suitable for exercising hepatectomy, while the use of liver transplantation is limited by the lack of organs due to the insidious onset of early hepatocellular carcinoma symptoms [3, 4]. The multi-component and multi-targeted action features of traditional Chinese medicine not only inhibit and stabilize tumor development, but also improve patients' immune function and lessen the cytotoxic effects of radiotherapy, which has good potential for development [5]. As a result, the comprehensive therapeutic approach combining traditional Chinese medicine has played an increasingly important role in recent years.

In China, a material known as MF is both a medication and a functional food. Chinese medicine contended that MF is associated with the spleen, stomach, and large intestine meridians and has the effect of warming the middle and astringent intestines, promoting Qi flow, and removing food. It is primarily used to treat stomach and abdominal distension and pain, less appetite, and vomiting [6].

Numerous MF active components have been shown to have anticancer properties by recent study [7], however the exact mechanism behind these effects is yet unknown. A crucial step in advancing the modernisation of Chinese medicine is the development of network pharmacology, a branch of pharmacology that places an emphasis on a comprehensive and systematic examination of the relationship between drug and disease action and the use of network methodologies to do so [8, 9]. This paper, which is based on the concept of network pharmacology analysis, aims to investigate the potential of the MF active ingredient for the treatment of hepatocellular carcinoma and to attempt to evaluate the mechanism of action in order to serve as a guide for future research.

1.1 Materials

The dried *Myristica fragrans* Houtt. kernel was purchased from Zhuhai Pharmaceutical Market, which authenticated by Zhang Runrong, senior engineer of traditional Chinese medicine, College of Pharmacy and Food Science, Zhuhai College of Science and Technology, Zhuhai, Guangdong Province, China, and the plant samples are stored in the Zhuhai College of Science and Technology, College of Pharmacy and Food Science Traditional Chinese Medicine Preservation Center, sample number 20221201. DMEM high-sugar medium (C11965500BT) was purchased from Gibco Co., Ltd. (California, USA). Fetal Bovine Serum (FBS) was purchased from Procell Life Science & Technology Co., Ltd. (Wuhan, China). Penicillin - streptomycin solution (SV30010) was purchased from HyC lone Co., Ltd. (Logan, USA). Cell Counting Kit-8 (CCK8, C0038) was purchased from Beyotime Biotechnology Co., Ltd. (Shanghai, China). Annexin V/PI kit (CA1020) was purchased from Solarbio Co., Ltd. (Beijing, China).

1.2 Drug Chemical Composition and Target Screening

The TCMSP database and analysis platform (http://tcmspw.com/tcmsp.php) was searched for " *Myristica fragrans* Houtt." [10], and combined with the literature research and database screening recommendations (Liang et al., 2021; Yang et al., 2020), oral bioavailability (OB) ≥ 30 % and drug-like (DL) ≥ 0.18 were used as the screening conditions to obtain the chemical constituents of MF, OB ≥ 30 % and drug-like (DL) ≥ 0.18 were used as screening conditions to obtain the chemical constituents of MF; the TCMSP database was used to search for the corresponding targets of the candidate chemical constituents, and the Uniprot database (http://www.uniprot.org/) was used to convert the target genes into a standardized form (genesymbol); the chemical composition targets were supplemented using the Swiss Target Prediction database (http://www.swisstargetprediction.ch/).

1.3 Disease Associated Target Acquisition

The GeneCards database (https://www.genecards.org/) was searched for "hepatocellular carinoma" to obtain hepatocellular carcinoma-associated gene targets [11].

1.4 Acquisition of Drug-Disease Targets and Construction of Network Relationship Map

Using the Venny 2.1 online mapping platform (https://bioinfogp.cnb.csic.es/tools/venny/index.html), we input the action targets of MF chemical components and hepatocellular carcinoma respectively and take the intersection operation to obtain the common action targets of MF and hepatocellular carcinoma; using Cytoscape 3.7.1 software to construct the "drug component-disease target" network, and use the Network Analyzer function to calculate the topological parameters of the network, and analyze the effect of MF on hepatocellular carcinoma according to the degree, betweenness (BC) and closeness (CC). Analysis of the core components of MF for the treatment of hepatocellular carcinoma.

1.5 Construction and Analysis of PPI Network

Upload the intersecting targets of MF and hepatocellular carcinoma to STRING database (https://string-db.org/) for PPI network analysis [12], set "species" to "Homo sapiens" and "minimum interaction threshold" to "medium confidence > 0.4". The "minimum interaction threshold" was set to "medium confidence > 0.4," and the isolated nodes were hidden, and finally the results were imported into cytoscape 3.7.1 software to construct the "target protein interaction relationship" network. The results were imported into cytoscape 3.7.1 software to build the "target protein interaction relationship" network diagram, and the Network Analyzer tool was used to filter the key targets based on the degree values.

1.6 Biological Process and Pathway Enrichment Analysis

The core targets obtained from PPI network analysis were imported into DAVID data analysis platform [13] (https//david.ncifcrf.gov/) for gene ontology (GO) functional enrichment analysis and genome encyclopedia (KEGG) pathway analysis, and the 10 biological processes and 15 signaling pathways with the highest significance were screened based on P-values and plotted in column and bubble enrichment maps. The enriched targets on the pathways were matched with the corresponding active components, and the data were imported into cytoscape 3.7.1 software to construct a "component-target-pathway" network relationship map.

1.7 Molecular Docking Verification

After network topology analysis, the obtained core components of MF for hepatocellular carcinoma were molecularly docked to the core acting targets of PPI network. The 3D structure of the core target protein was obtained in the PDB database [14] (http://www.rcsb.org/), the target protein water molecule and ligand were removed using PyMOL 2.4 software, and imported into AutoDock Tools 1.5.6 for hydrogenation and charge assignment, and after setting the docking box center using the Grid module, running Autodock 4.2 was used to perform molecular docking. The molecular docking effect was evaluated by the minimum binding energy, and the molecular docking effect was processed using PyMOL 2.4.

1.8 Experimental Verification of Cell Biology and Pharmacology

1.8.1 Preparation of MF.

Crush the dried Myristica fragrans Houtt. kernel, add 10 times of 70 % ethanol of powder quality, heat and reflux for 3 times, each time for 2 hours, combine the filtrate, decompress and concentrate until there is no ethanol smell, and evaporate to dry to obtain the ethanol extract of MF.

1.8.2 Cell Culture.

Use DMEM medium, add 10 % FBS, 100 mg/mL streptomycin and 100 U/mL penicillin, culture for 24 hours at 37 ℃ and 5 % CO_2, and take logarithmic growth cells for experiment.

1.8.3 Determination of the Effect of MF on the Proliferation of HepG2 Cells.

The impact of MF on the multiplication of HepG2 cells was distinguished by CCK-8 method. HepG2 cells were treated with 1×10^4 cells/well density and inoculated in 96-well plate with the incubator for 24 hours. With dimethyl sulfoxide (DMSO) as solvent, the preparation concentration is 0 μg/mL、50 μg/mL、100 μg/mL、200 μg/mL MF dosing solution, treat the cells with the above MF, put 96-well plate into a 37 ℃, 5 % CO_2 incubator for 24 hours, add 10 % CCK-8 to continue to culture for 1 hour,

measure the absorbance value at 450 nm with an enzyme marker, and calculate the cell survival rate. The calculation formula is:

$$\text{Relative survival rate of cells (\%)} = \frac{At-Ab}{Ac-\text{Ab}} \times 100\% \quad (1)$$

In the formula, *At* is the OD value of the experimental group; *Ac* is the OD value of the control group, and *Ab* is the OD value of the blank group.

1.8.4 Determination of the Effect of MF on HepG2 Cell Apoptosis.

The cell apoptosis was measured with FITC (fluorescein isothiocyanate) annexin V apoptosis detection kit. HepG2 cells (2×10^5 cells/well) were incubated in a 6-well plate, and then at the end of treatment, the cells under different treatments were separated with trypsin without EDTA, and washed with PBS solution. After centrifugation, plasma cells were suspended in binding buffer. Add annexin V-FITC staining solution (5 μL) and PI staining solution (5 μL) after incubation at room temperature for 10 min, the cells were analyzed by flow cytometry Cyto FLEX (Beckman, Indianapolis, US).

1.9 Statistical Analysis

Data are shown as mean ± SEM, and statistical analysis was performed using Origin 2021, Graphpad Prism 8, and EXCEL 2019. To compare the differences between multiple groups, a one-way analysis of variance (ANOVA) was performed in this software. $P < 0.05$ indicates statistical significance.

2 Results

2.1 MF Active Ingredients and Target Acquisition

Table 1. The active ingredient OD MF.

Numbe	Molecule ID	Ingredient	Molecular Fomula	OB/%	DL
RDK1	MOL000358	Beta-Sitosterol	$C_{29}H_{50}O$	31.32	0.26
RDK2	MOL007920	Dihydroguaiaretic acid	$C_{20}H_{26}O_4$	31.32	0.26
RDK3	MOL009243	Isoguaiacin	$C_{20}H_{24}O_4$	48.78	0.31
RDK4	MOL009254	Galbacin	$C_{20}H_{20}O_5$	61.0	0.53
RDK5	MOL009255	Licarin B	$C_{20}H_{20}O_4$	53.11	0.40
RDK6	MOL009259	Kudos	$C_{21}H_{20}Cl_2O_3$	45.06	0.38
RDK7	MOL009263	Saucernetindiol	$C_{20}H_{24}O_5$	41.85	0.32
RDK8	MOL009264	Tetrahydrofuroguaiacin B	$C_{20}H_{24}O_5$	62.86	0.32
RDK9	MOL009265	threo-austrobailignan-5	$C_{19}H_{20}O_2$	49.49	0.32

In the TCMSP database, OB ≥ 30% and DL ≥ 0.18 were used as screening conditions, and a total of 9 active ingredients were obtained, as shown in Table 1. The obtained active ingredients corresponding targets were standardized against the UniProt database, and the Swiss Target Prediciton database was used to supplement the active

ingredient action targets, and the two groups of target information were combined and duplicate action targets were deleted. In total, 366 active ingredient targets of MF were screened, as shown in Table 1.

2.2 Screening for Component-Disease Crossover Gene Targets

The GeneCards data were searched for "hepatocellular carinoma" and 5,261 hepatocellular carcinoma-associated targets were obtained by deleting the duplicate targets. Using the Venny 2.1 online graphical analysis platform, we entered the targets of MF and hepatocellular carinoma, and obtained 241 targets of MF in hepatocellular carinoma, and drew a Venn diagram, in Figure 1.

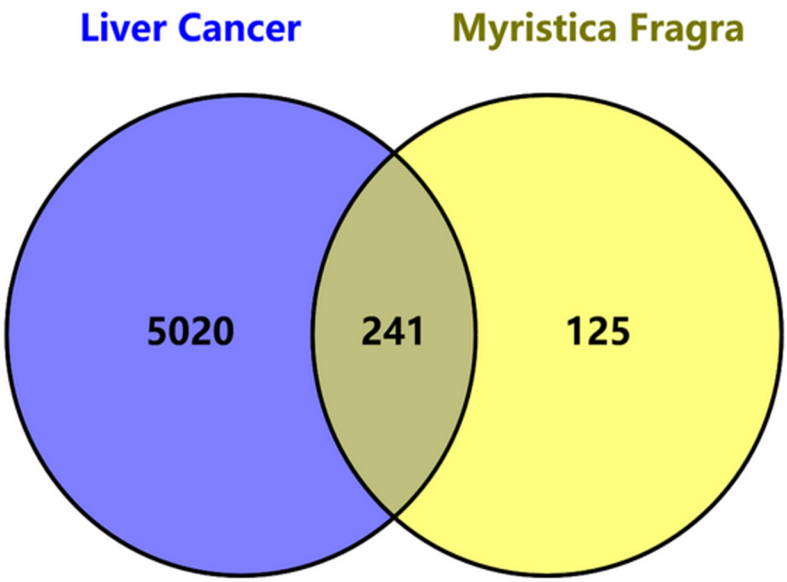

Fig. 1. Venn diagram of the active ingredient targets of MF and liver cancer targets.

2.3 Drug-Disease Target Network Construction and Core Action Component Screening

The 241 intersecting targets of the nine active ingredients of MF and hepatocellular carinoma were imported into Cytoscape 3.7.1 software to build a "drug-disease-target" network (Figure 2). The network was analyzed topologically using the Network Analyzer function of the software, and the results showed that the average degree of the network was 3.49; the average median centrality (BC) was 0.0103; the average closeness centrality (CC) was 0.34, indicating that MF presents multi-component and multi-

target characteristics in hepatocellular carcinoma. Acid, Isoguaiacin, Licarin B, and Kudosthreo-austrobailignan-5 are the core components acting on hepatocellular carcinoma targets (Table 2).

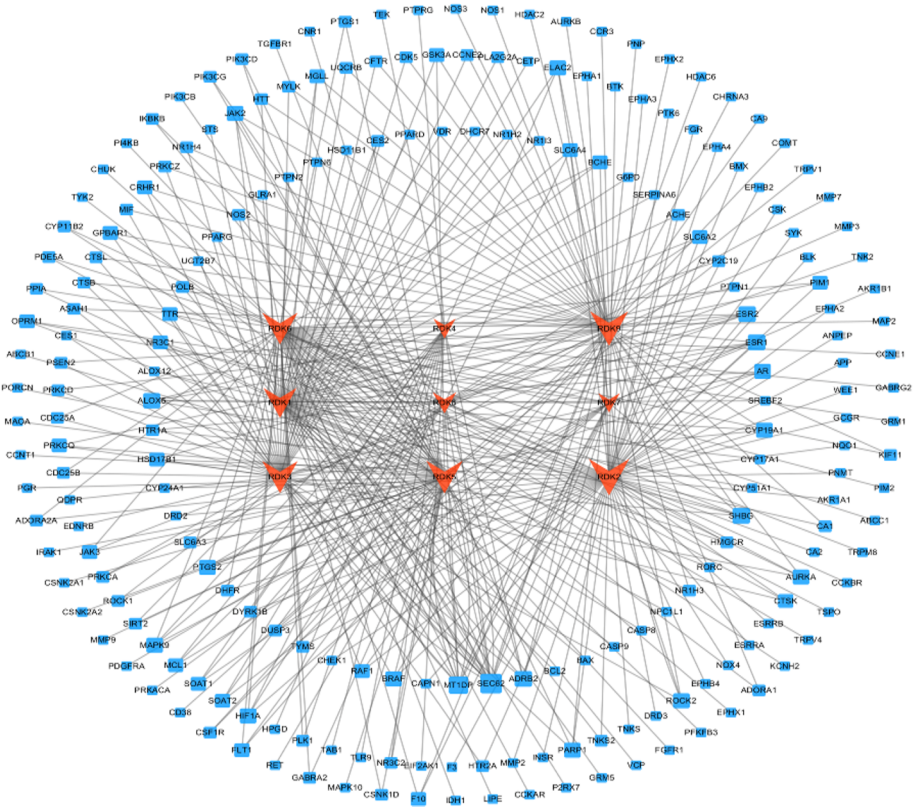

Fig. 2. MF component-liver cancer target action network relationship diagram. Note: Red arrow nodes represent MF components (indicated by number), blue square nodes represent target sites, and the larger area of the icon color represents the stronger action relationship.

Table 2. Topological analysis of MF component-hepatocellular carcinoma target network.

Ingredient	Degree	BC	CC
Dihydroguaiaretic acid, RDK2	66	0.35014216	0.42205323
threo-austrobailignan-5, RDK9	60	0.22884143	0.41111111
Licarin B, RDK5	55	0.24172373	0.39642857
Isoguaiacin, RDK3	53	0.17033364	0.40072202
Kudos, RDK6	52	0.24276327	0.39928058
Beta-Sitosterol, RDK1	47	0.22139841	0.38541667
Tetrahydrofuroguaiacin B, RDK8	22	0.06310324	0.36156352
Galbacin, RDK4	18	0.06666723	0.35691318
Saucernetindiol, RDK7	17	0.02727262	0.35463259

2.4 PPI Analysis and Core Protein Interaction Network Screening

The above 241 targets were imported into STRING database for PPI analysis, and the "minimum interaction threshold" was set to "medium confidence > 0.4", and the isolated nodes were hidden to obtain the protein interaction network of hepatocellular carcinoma targets of MF. The results showed that the median degree of PPI network was 18, so the core targets were selected with a median value greater than or equal to 2 times the median value of PPI network. The network topology analysis showed that AKT1, SRC and EGFR had the highest degree value in the network, and it was assumed that these targets were the core targets of MF for hepatocellular carcinoma treatment (Figure 3).

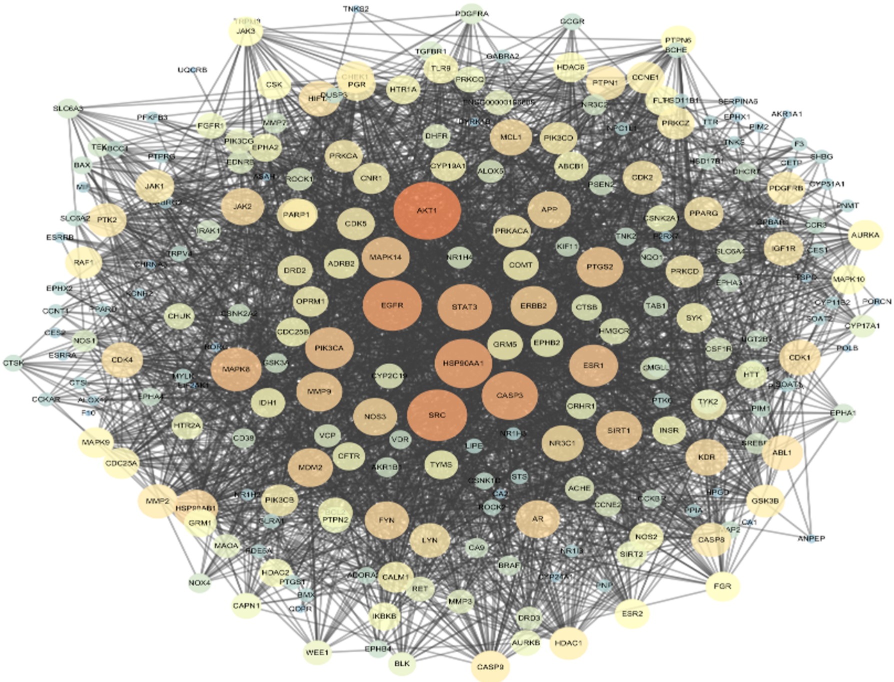

Fig. 3. Protein interaction network relationship of MF targets for hepatocellular carcinoma treatment

2.5 Biological Process and Pathway Enrichment Analysis

The 42 targets of the above PPI core network were imported into David's data analysis platform for gene ontology (GO) function enrichment analysis, and 346 biological processes and signaling pathways were enriched by using P < 0.05 as the screening condition. The 10 biological processes, cellular componet and molecular function processes with the most significant effects were screened in ascending order of P value and plotted as bar graphs in Figure 4. Growth factor receptor signaling pathway, tyrosine phosphorylation, drug response, positive regulation of cell proliferation, etc.; the main cellular

components affected were nuclear, cytoplasmic, mitochondrial and other regions; the main cellular functions involved were protein binding, tyrosine kinase activity, ATP binding, etc.

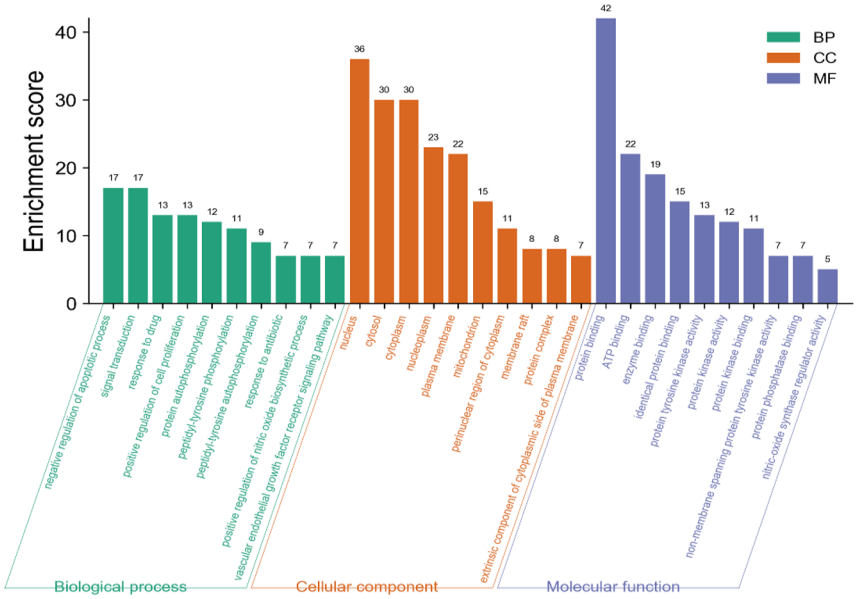

Fig. 4. GO enrichment analysis of the core targets of active ingredients of MF.

2.6 Component-Target-Signaling Pathway Network Construction

KEGG signaling pathway analysis was performed on the above 42 core targets, and 82 signaling pathways were obtained with $P < 0.05$ as the screening condition, and the most significant 15 signaling pathways were screened in ascending order of P value and plotted in dotted bubble diagrams, see Figure 5. The analysis results showed that the potential signaling pathways acted by the active ingredients of MF are PI3K-Akt signaling pathway, VEGF signaling pathway, FoxO signaling pathway and ErbB signaling pathway.

The 15 most significant signaling pathways obtained by KEGG enrichment analysis may have potential anti-hepatocellular carcinoma effects. Therefore, the KEGG analysis platform was used to obtain the targets corresponding to the above 15 signaling pathways and to find the corresponding active ingredients of MF, to construct the "ingredient-target-signaling pathway" network of MF for the treatment of hepatocellular carcinoma. The topological analysis showed that the active ingredients of MF with the highest network value were cis-austrobin (Kudos, RDK6), trans-austrobailig-nan-5 (RDK9), Licarin B (RDK5), dihydroguaiaretic acid (Dihydroguaiaretic acid, RDK6), and the active ingredient of the network. Dihydroguaiaretic acid (RDK2) and isoguaiacin (RDK3), indicating that these components are the core components of the hepatocellular carcinoma signaling pathway, which is consistent with the results of the drug-

disease-target screening of the core components of action. The highest targets in the network were PIK3CA, AKT1, GSK3B, EGFR, SRC, MAPK8, etc., indicating that the above targets are the core targets of MF in the hepatocellular carcinoma signaling pathway, which is consistent with the results of protein interaction cores screened in the PPI network (Figure 6).

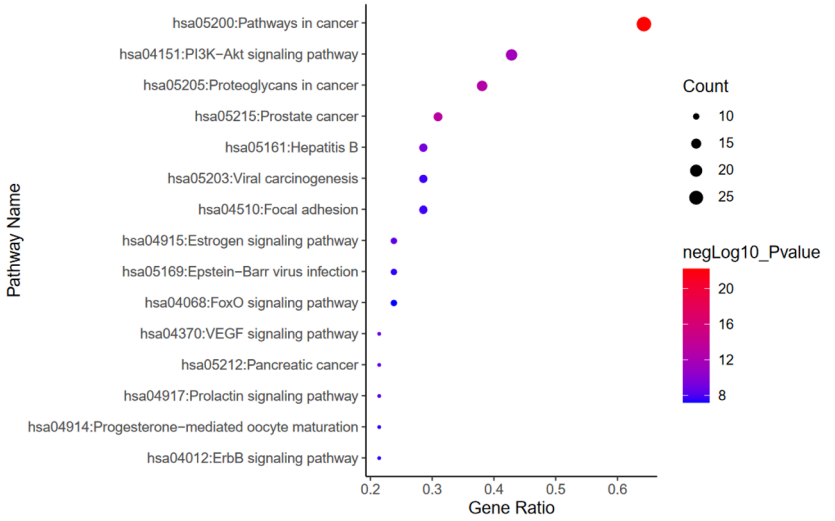

Fig. 5. Enrichment analysis of KEGG

2.7 Molecular Docking Verification

In order to verify the effects of MF active ingredients on the core targets of hepatocellular carcinoma, the five MF active ingredients with the highest topological values in the "ingredient-target" network analysis, cis-cypermethrin, trans-auxin, ricarin B, dihydroguaiaretic acid and iso-guaiaretic acid, were selected and the three targets with the highest topological values in the PPI network, AKT1 SRC and EGFR were molecularly docked using Autodock 4.2 software. The effect of docking was evaluated by the minimum binding energy (Binding Energy), theoretically, when the binding energy is < 0, it indicates that the ligand molecule can spontaneously bind to the receptor molecule, while the larger the absolute value of the binding energy [15], the stronger the binding effect, and when the absolute value of the binding energy > 5.0, it indicates a strong binding effect. The corresponding ligands of the targets were selected as controls, and the molecular docking results are shown in Table 3. The results indicate that the five active components of MF can spontaneously bind to the three targets, and most of the components have strong binding effects with the targets; while Licarin B, trans-auxin 5, and isoguaiacrine have higher docking scores with the three targets, indicating that the three components have the strongest effects on the targets. The molecular docking pattern diagram is shown in Figure 7.

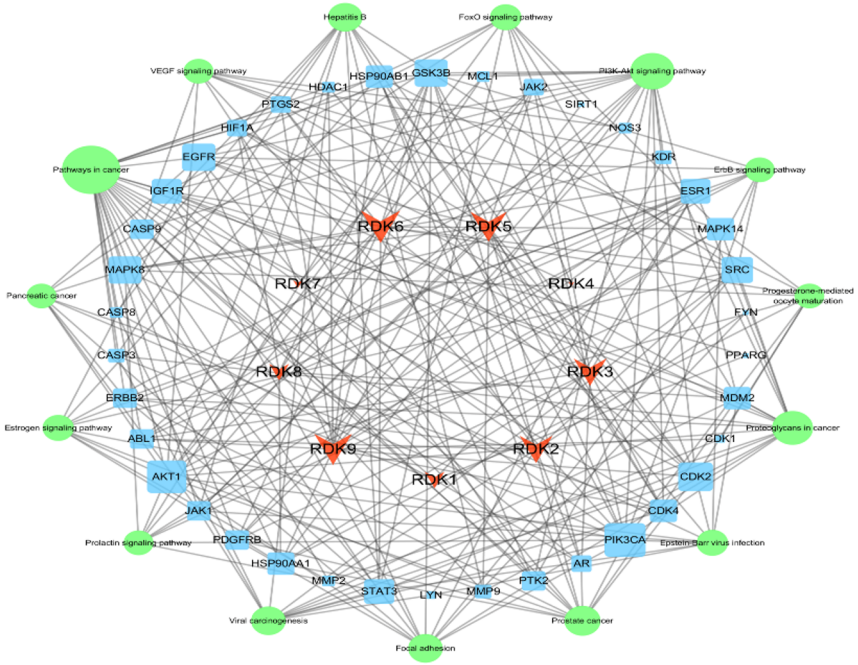

Fig. 6. MF active ingredient-liver cancer target-signaling pathway network relationship diagram.

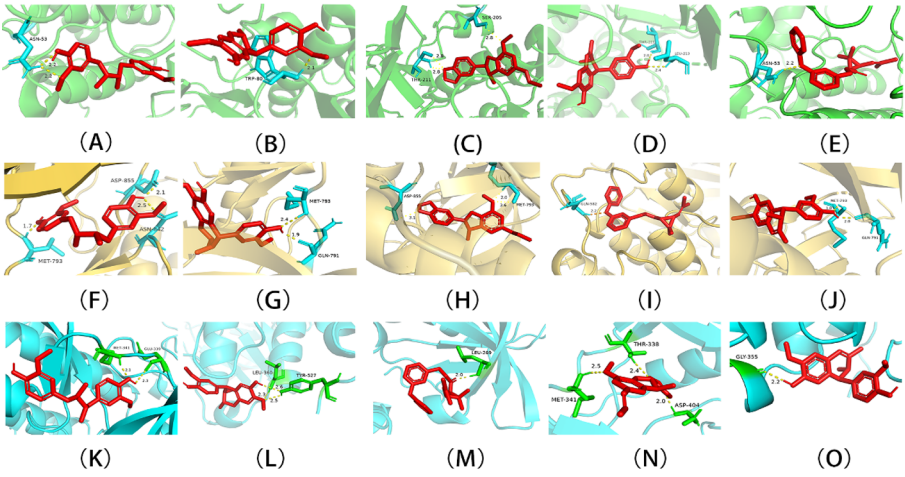

Fig. 7. Effect of GPP pretreatment on apoptosis (A) and mitochondrial membrane potential (B) in injury models. Note: A. Dihydroguaiaretic acid–AKT1; B. Isoguaiacin–AKT1; C. Licarin B–AKT1; D. Kudos–AKT1; E. Threo-austrobailignan-5–AKT1; F. Dihydroguaiaretic acid–EFFR; G. Isoguaiacin–EGFR;H. Licarin B–EGFR; I. Kudos–EGFR; J. threo-austrobailignan-5–EGFR; K. Dihydroguaiaretic acid-SRC; L. Isoguaiacin–SRC; M. Licarin B–SRC;N. Kudos–SRC; O. Threo-austrobailignan-5–SRC.

Table 3. Molecular docking results of active ingredients of MF and core targets of action

Compound	Minimum binding energy (kcal/mol)		
	AKT1	SRC	EGFR
Ligand (Positive control)	-9.51	-7.96	-10.50
Dihydroguaiaretic acid	-6.73	-4.37	-6.24
Isoguaiacin	-6.11	-6.65	-7.14
Licarin B	-8.36	-7.26	-7.68
Kudos	-8.02	-5.72	-5.95
threo-austrobailignan-5	-7.16	-7.08	-7.11

2.8 Experimental Verification of Cellular Pharmacology

In order to further verify the anti-hepatoma effect of MF and the effect on the proliferation of HepG2 cells, the results are shown in Figure 8. Compared with the control group, the cell survival rate of each treatment group was significantly diminished ($p < 0.05$), of which 200 μg/mL treatment group with MF the survival rate of cells was decreased to 57.04 ± 4.51 %.

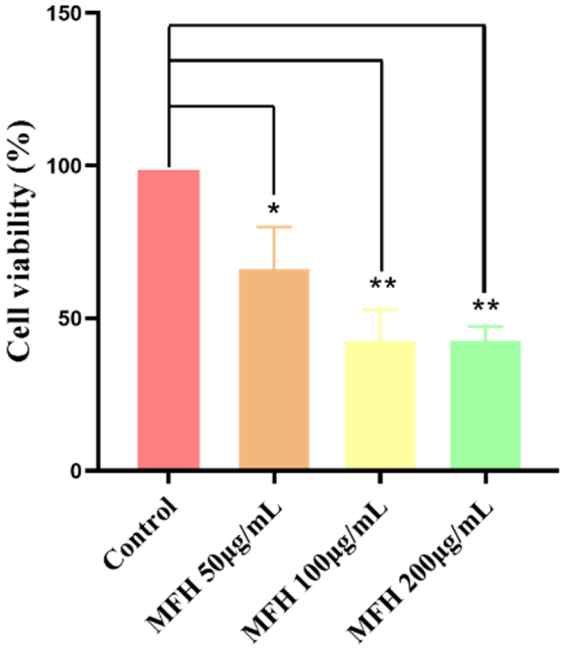

Fig. 8. Effects of active components of MF on the survival rate of HepG2 cells. $^{*}p < 0.05$, $^{**}p < 0.01$, versus Control group.

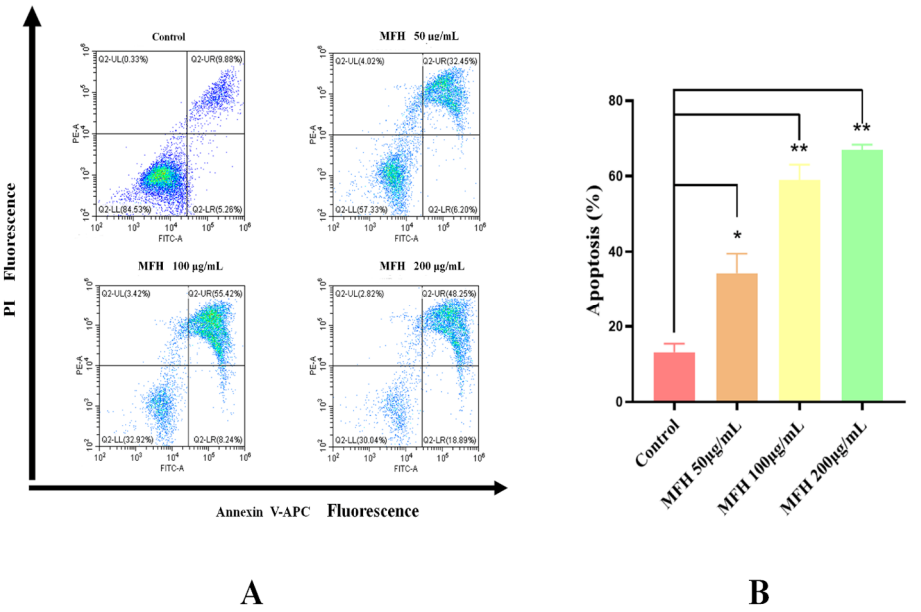

Fig. 9. Effect of MF on HepG$_2$ cell apoptosis. (A) Use of flow cytometry to detect the level of apoptosis; (B) Statistics of apoptosis level. $^{*}p < 0.05$, $^{**}p < 0.01$, versus Control group.

The effect of MF on the apoptosis of HepG2 cells was further investigated by flow cytometry. The results are shown in Figure 9. Compared with the control group, the apoptosis rate of each treatment group was significantly increased ($p < 0.05$), of which 200 μg/mL treatment group with MF the cell apoptosis rate was increased to 66.91 ± 1.46 %

3 Discussion

Hepatocellular carcinoma is a malignant tumor with a generally high prevalence worldwide and also one of the leading causes of tumor-related deaths, and with the changes in people's life, work habits, dietary patterns, and environment, the incidence of hepatocellular carcinoma is on the rise in most countries and regions around the world [16]. At present, the comprehensive treatment combining surgery, radiotherapy, targeted drugs and herbal therapy significantly benefits hepatocellular carcinoma patients more than single treatment, and the multi-component and multi-target action characteristics of herbal medicine not only can reduce the tumor volume and prolong the survival time of patients, but also can improve the adverse symptoms caused by other treatment modalities, which has good potential for development [17, 18]. In this paper, based on a network pharmacology approach, we found that the active components of MF have potential effects in the treatment of hepatocellular carcinoma, among which dihydroguaiaretic acid, iso-guaiacrine, licarine B, cis-cypermethrin, and trans-auxin 5 are the core components acting on the targets of hepatocellular carcinoma. Dihydroguaiaretic acid

has been shown to have in vitro antitumor activity [19]. Isoguaiacrine helps to maintain glutathione and superoxide dismutase levels in hepatocytes thus exerting antioxidant and hepatocyte-protective effects [20], and ricarin B exerts anti-inflammatory effects by inhibiting the NO synthesis pathway [21].

The screening results of PPI network analysis showed that there are 43 core targets of MF active ingredients acting in hepatocellular carcinoma, mainly AKT1, SRC, EGFR, CASP3, STAT3 and so on. Among them, EGFR and AKT1 are components of the PI3K-AKT signaling pathway, and when these two targets are activated, they then act on downstream substrates such as MTOR and P70S6K, which eventually accelerate cellular protein production and growth, thus accelerating the process of tumor progression [22]. STAT3 is a key regulatory gene of the JAK2/STAT3 signaling pathway, and high expression of STAT3 has been widely reported to be closely related to hepatocellular carcinoma because it can rapidly regulate gene transcription levels and thus accelerate tumor cell appreciation and invasion, and it has been widely reported that high expression of STAT3 is associated with hepatocellular carcinoma because it can rapidly regulate gene transcription levels to accelerate tumor cell appreciation and invasion, and inhibit tumor cell apoptosis by upregulating Bcl-xl, Mcl-1, cyclins and downregulating P53 gene expression [23]. Based on the above analysis, it is hypothesized that the active ingredients of MF have the potential to exert anti-tumor proliferation, invasion and apoptosis-promoting effects by inhibiting the above target proteins.

Further, GO and KEGG analysis showed that the biological processes of the core targets were mainly enriched in the negative regulation of apoptotic process, vascular endothelial growth factor receptor signaling pathway, tyrosine phosphorylation, and positive regulation of cell proliferation, which was also consistent with the PPI analysis; KEGG analysis showed that the potential pathways of MF for hepatocellular carcinoma were PI3K-AKT, FoxO, and VEGF FoxO is a downstream effector pathway of PI3K-AKT, and an over-activated PI3K-AKT pathway in tumor cells will inactivate FoxO and prevent FoxO-mediated cell proliferation arrest and induction of cell death to achieve cell survival [24]. The formation of a hypoxic microenvironment will induce the activation of the hypoxia-inducible factor HIF and act on VEGF, which will activate multiple downstream signaling pathways, ultimately leading to cell proliferation, migration and neoangiogenesis [25].

HepG2 cell line was chosen in this investigation to confirm the anti-tumor action of MF in vitro since the emergence of tumor is closely related to the imbalance of cell proliferation and apoptosis. The anti-hepatoma activity of MFH increased with drug concentration, according to the results of the CCK-8 method; the Annexin V-FITC/PI double staining method was utilized to determine that MFH could considerably trigger the apoptosis of $HepG_2$ cells at the chosen administration concentration. The cytological tests mentioned above proved that MF has anti-hepatoma potential.

4 Conclusion

In conclusion, based on the network pharmacological analysis method, this paper explores the action potential of the active ingredient of MF in the treatment of

hepatocellular carcinoma and analyzes the potential mechanism of its action in hepatocellular carcinoma, which provides a reference for the future development of Chinese herbal medicine for the treatment of hepatocellular carcinoma, and the subsequent experimental verification of the above predicted results is also needed.

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