基于网络药理学的香菊制剂治疗鼻炎、鼻窦炎的药效物质基础研究

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摘要: 本研究拟通过构建“成分−靶点−通路”的多层次网络整合分析, 探讨香菊制剂 (香菊片、香菊滴剂) 治疗鼻炎、鼻窦炎的药效物质及其作用机制。采用高效液相色谱−四级杆飞行时间质谱 (HPLC-QTOF/MS) 技术首次鉴定出香菊制剂中137种化学成分, 对其中59种潜在活性成分进行网络药理学分析, 结果表明咖啡酸、洋川芎内酯F、迷迭香酸、藁本内酯、升麻素苷、蒙花苷、木兰脂素、木犀草素、洋川芎内酯I及没食子酸等10种成分可作用于肿瘤坏死因子 (tumor necrosis factor, TNF)、白细胞介素1B (interleukin 1B, IL1B)、蛋白激酶B (protein kinase B, AKT1)、血管内皮生长因子A (vascular endothelial growth factor A, VEGFA)、信号传导及转录激活蛋白-3 (signal transducer and activator of transcription 3, STAT3) 等核心靶点, 通过调节晚期糖基化产物−晚期糖基化终末产物受体 (advanced glycosylation end products-receptor of AGEs, AGE-RAGE)、TNF、核转录因子*κ*B (nuclear factor kappa B, NF-*κ*B)、环磷酸鸟苷−蛋白激酶G (cyclic guanosine monophosphate-protein kinase G, cGMP-PKG) 等信号通路进而治疗鼻炎、鼻窦炎。分子对接结果显示, 以上10种化学成分与5个关键靶点间均具有良好的结合性能, 说明这10种化学成分为香菊制剂潜在药效物质。综上, 本研究初步阐明了香菊制剂治疗鼻炎、鼻窦炎的药效物质及其作用机制, 为香菊制剂的临床应用提供了理论基础。

关键词: 香菊制剂; 高效液相色谱−四级杆飞行时间质谱; 网络药理学; 鼻炎; 鼻窦炎

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Study on the pharmacodynamic material basis of Xiangju Preparations in the treatment of rhinitis and sinusitis based on network pharmacology

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Abstract: In order to clarify the pharmacodynamic substances and mechanism of Xiangju Preparations (Xiangju Tablets, Xiangju Drops) in the treatment of rhinitis and sinusitis, the multi-level network integration analysis of "ingredients-targets-pathways" was conducted. 137 chemical constituents were identified in Xiangju Preparations by high pressure liquid chromatography-quadrupole-time of flight mass spectrometry (HPLC-QTOF/MS) for the first time. Network pharmacology analysis was performed on 59 potential active components. The results of network pharmacology analysis demonstrated that the medicinal ingredients in Xiangju Preparations included caffeic acid, senkyunolide F, rosmarinic acid, ligustilide, prim-*O*-glucosylcimifugin, linarin, magnolin, luteolin, senkyunolide I and gallic acid. These ingredients act on the crucial targets of tumor necrosis factor (TNF), interleukin 1B (IL1B), protein kinase B (AKT1), vascular endothelial growth factor A (VEGFA), signal transducer

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and activator of transcription 3 (STAT3) and participate in the regulation of advanced glycosylation end products-receptor of AGEs (AGE-RAGE), TNF, nuclear factor kappa B (NF-κB), and cyclic guanosine monophosphate-protein kinase G (cGMP-PKG) signaling pathways to effectively treat rhinitis and sinusitis. The excellent binding performance between above 10 active components and 5 key target proteins was further confirmed by molecular docking, indicating that these 10 ingredients are pharmacodynamic substances of Xiangju preparations. In conclusion, this study preliminarily clarified the effective components and mechanism of Xiangju preparations in the treatment of rhinitis and sinusitis, and provided a theoretical basis for the clinical application of Xiangju preparations.

Key words: Xiangju preparation; HPLC-QTOF/MS; network pharmacology; rhinitis; sinusitis

鼻炎、鼻窦炎均为临床上常见的鼻科疾病。在我国, 鼻炎和鼻窦炎的发病率持续上升, 严重影响了人民的生活质量[1,2]。目前, 临床上常用抗生素、抗组胺药和糖皮质激素等药物治疗鼻炎、鼻窦炎, 但该类药物疗效欠佳, 并伴有中枢神经系统抑制、心脏毒性等不良反应[3,4]。中药则因其安全性高、作用广泛等特点, 一直以来是研究的热点, 在治疗鼻部疾病方面具有较大的潜能。

香菊片和香菊滴剂 (统称为香菊制剂) 是根据同一民间验方研制的不同剂型的中成药, 该方共由9味中药组成, 方中化香树果序活血化瘀, 为君药; 夏枯草和野菊花可去痰散结, 泻火消肿, 均为臣药; 川芎、白芷、辛夷、防风、黄芪、甘草益气扶正, 生肌托疮, 同为佐药; 甘草具有调和诸药的作用, 同时也是本方的使药。全方合用具有辛散祛风、清热解毒的功效, 对于鼻炎、鼻窦炎的治疗具有积极作用[5,6]。

目前, 香菊片已应用于临床治疗鼻炎、鼻窦炎, 但关于香菊片的研究主要集中在临床疗效方面[7], 缺乏其有效成分及作用机制方面的研究, 阻碍了该药的进一步开发与应用。香菊滴剂与香菊片的提取工艺虽然相同, 但后续制备工艺的差异也会影响原有活性成分的种类和含量, 从而影响药物的质量和疗效。因此, 本研究采用高效液相色谱-四级杆飞行时间质谱 (HPLC-QTOF/MS)技术与网络药理学方法相结合的综合策略, 预测了香菊制剂的药效物质, 探讨了香菊制剂治疗鼻炎、鼻窦炎的潜在作用机制, 以期为香菊制剂的临床应用和质量标准的提升提供理论依据。

材料与方法

仪器 1260 Infinity II型 HPLC (美国安捷伦公司); QTOF-5600 型 MS (AB SCIEX 公司); KQ-500DB 超声波清洗器 (昆山市超声仪器有限公司); MT-XS105 分析天平 (瑞士 Mettler Toledo 公司); MTAB135-S 分析天平 (日本岛津公司); TDZ4 WS 型离心机 (湖南湘仪实验室仪器开发有限公司)。

材料与试剂 香菊片 (20210407)、香菊滴剂 (20210407) 均由陕西香菊药业集团有限公司提供; 没食子酸 (110831-201906)、升麻素苷 (111522-201913)、甘草苷 (111610-201908)、芦丁 (100080-201811)、鞣花酸 (111959-201903)、迷迭香酸 (111871-202007)、蒙花苷 (111528-201911)、木兰脂素 (110882-201909)、甘草酸铵 (110731-201720) 均购自中国食品药品检定研究院; 木犀草素 (CHB201228)、升麻素 (CHB170220)、芹糖异甘草苷 (CHB201126) 均购自成都克洛玛生物科技有限公司; 水合氧化前胡素 (P14O10F100115)、氧化前胡素 (Y02N9S71244) 均购自上海源叶生物科技有限公司; 1,5-二咖啡酰基奎宁酸 (MUST-17022608) 购自成都曼思特生物科技有限公司; 甲醇 (色谱纯, 美国 Sigma-Aldrich 公司); 乙腈 (色谱纯, 美国 Fisher 有限公司); 甲酸 (色谱纯, 天津市科密欧化学试剂有限公司); 纯净水 (杭州娃哈哈有限公司)。

化学成分的鉴定

色谱条件 色谱柱: Waters SymmetryShield™ RP18 (150 mm × 4.6 mm, 3.5 μm); 流动相: 0.1% 甲酸水溶液 (A)−乙腈 (B), 洗脱程序: 0～3 min, 100% A; 3～10 min, 100%～97% A; 10～15 min, 97%～95% A; 15～18 min, 95% A; 18～25 min, 95%～90% A; 25～30 min, 90%～88% A; 30～50 min, 88%～80% A; 50～70 min, 80%～73% A; 70～95 min, 73%～65% A; 95～110 min, 65%～60% A; 110～120 min, 60%～55% A; 120～125 min, 55%～5% A; 流速: 0.4 mL·min^{-1}; 柱温: 30 ℃; 进样量: 5 μL。

质谱条件 离子源: 电喷雾离子源; 检测模式: 正、负离子检测; 离子喷雾电压为5 500 V和−4 500 V; 离子源温度: 550 ℃; 喷雾辅助气体: 氮气; 喷雾气压力: 50 psi (1 psi ≈ 6.9 kPa); 辅助加热气压力: 50 psi; 气帘气压力: 30 psi; TOF MS 模式下扫描范围为 *m/z* 50～1 500 Da; 去簇电压: 80 V (正离子)/−80 V (负离子); 碰撞能: 10 V (正离子)/−10 V (负离子); TOF MS/MS 模式下扫描范围为 *m/z* 50～1 500 Da; 去簇电压: 80 V (正离子)/−80 V (负离子); 碰撞能: 30 V (正离子)/−30 V (负离子)。

香菊片供试品溶液的制备　取本品10片, 除去包衣, 研细, 取约2 g, 精密称定, 置具塞锥形瓶中, 精密加入甲醇20 mL, 称定重量, 超声30 min, 放冷, 再次称定重量, 用甲醇补足减失的重量, 摇匀, 离心 (4 000 r·min^{-1}, 10 min), 取上清液, 过0.22 μm滤膜, 即得。

香菊滴剂供试品溶液的制备　精密吸取本品2 mL, 置于20 mL量瓶中, 加甲醇适量, 超声30 min, 放冷, 甲醇定容至刻度, 摇匀, 离心 (4 000 r·min^{-1}, 10 min), 取上清液, 过0.22 μm滤膜, 即得。

对照品溶液的制备　分别取没食子酸、升麻素苷、甘草苷、芦丁、鞣花酸、迷迭香酸、蒙花苷、木兰脂素、甘草酸铵、木犀草素、升麻素、芹糖异甘草苷、水合氧化前胡素、氧化前胡素、1,5-二咖啡酰基奎宁酸等15个对照品适量, 精密称定, 加甲醇溶解并定容, 配制成浓度均为30 μg·mL^{-1}的混合对照品溶液, 用于液质分析。

网络药理学分析　对鉴定的成分进行筛选, 满足以下任一情况即被纳入潜在活性成分: ①《中华人民共和国药典》2020年版中各味药材的指标性成分; ② 口服生物利用度 (oral bioavailability, OB) ≥ 30% 且类药性 (drug likeness, DL) ≥ 0.18 的成分; ③ 文献[8-27]报道的活性成分或含量较高的成分。利用TCMSP (http://tcmspw.com/tcmsp.php) 及Swiss Target Prediction (http://www.swisstarget prediction.ch/) 数据库获得成分靶点。在 OMIM (https://omim. org/)、TTD (http://db. idrblab.net/ttd/) 和Genecards (https://www.genecards.org/) 数据库中以“Rhinitis”和“Sinusitis”为关键词建立疾病靶点数据库。借助Venny 2.1在线分析工具获得香菊制剂成分与疾病的交集靶点, 即为香菊制剂治疗鼻炎、鼻窦炎的关键靶点。随后通过String (https://string-db.org/) 数据库 (限定物种为“Homo sapiens”) 分析关键靶蛋白间相互作用的关系网。利用Cluego插件对关键靶点进行通路富集分析, 最后采用Cytoscape软件构建“中药-成分-靶点”多层次网络图并进行拓扑学分析。

分子对接　从PDB (https://www1.rcsb.org/)数据库中获得受体蛋白3D结构, 利用PubChem (https://pubchem.ncbi.nlm.nih.gov/) 数据库获得配体成分的标准延迟格式文件 (SDF), 并通过Chem 3D 17.1软件将其转化为分子结构记录格式 (Mol2) 格式文件。设置合适的盒子中心与盒子格点参数, 将小分子配体可能结合的活性口袋位点包含, 使用Autodock分子对接模拟软件构建“受体-配体”结合模型并分析其结合性能。

结果

1　化学成分鉴定

运用建立的液质联用分析方法进行分析。针对结构相似的成分 (同一类的母核或者同系物) 可通过裂解生成相同子离子或发生相同中性丢失这一特点, 借助Peak View® V.2.2软件中的中性丢失过滤和子离子过滤技术, 对结构相关性化合物进行筛查, 在保留的有效信息中进行化合物结构的比对和预测。通过与对照品或文献报道过的成分的色谱保留行为、精确分子量、裂解规律及碎片离子信息进行比对, 在两种香菊制剂中均初步鉴定出137种化合物, 包括55个黄酮类化合物, 39个苯丙素类化合物, 11个有机酸类化合物, 9个萜类化合物, 8个苯酞类化合物, 6个色原酮类化合物和9个其他类化合物, 所有化合物质量误差均小于5 ppm, 鉴定结果及成分信息见表1。

2　香菊制剂治疗鼻炎、鼻窦炎的关键靶点分析

按照“网络药理学分析”方法中的筛选条件共得到59种潜在活性成分, 如表2所示。通过TCMSP及Swiss Target Prediction数据库查找成分的作用靶点, 以Probability > 0为限定条件共获得567个成分靶点。利用OMIM、TTD和Genecards等数据库获得了362个与鼻炎、鼻窦炎均有关的疾病靶点。采用Venny 2.1在线分析工具将成分靶点与疾病靶点取交集, 获得了63个香菊制剂治疗鼻炎、鼻窦炎的关键靶点。

Table 1　HPLC-QTOF/MS analysis of ingredients in Xiangju preparations. B: Angelicae Dahuricae Radix; C: Chuanxiong Rhizoma; F: Saposhnikoviae Radix; G: Glycyrrhizae Radix et Rhizoma; H1: Platycaryae Strobilaceae Fructus; H2: Astragali Radix; X1: Prunellae Spica; X2: Magnoliae Flos; Y: Chrysanthemi Indici Flos; *Identified with reference standard

No.	Identification	Formula	Ion adduction	Error /10^{-6}	t_R /min	Measured mass (*m/z*)	Fragment ion (*m/z*)	Source
1	Isogosferol	$C_{16}H_{14}O_5$	$[M+H]^+$	−2.2	72.88	287.091 4	147.042 9, 173.022 1, 202.395 0, 287.087 6	B
2	Oxypeucedanin hydrate*	$C_{16}H_{16}O_6$	$[M+H]^+$	−0.9	81.12	305.101 9	305.099 0, 203.031 0, 159.042 0, 147.042 2	B
3	Bergaptol	$C_{11}H_6O_4$	$[M-H]^-$	−0.3	83.18	201.019 3	117.036 1, 145.030 4, 173.024 5, 201.020 5	B
4	Oxypeucedanin*	$C_{16}H_{14}O_5$	$[M+H]^+$	−2.8	112.33	287.091 4	203.031 7, 147.042 1, 287.089 4, 202.396 2, 174.028 1	B

Continued

No.	Identification	Formula	Ion adduction	Error /10^{-6}	t_R /min	Measured mass (*m/z*)	Fragment ion (*m/z*)	Source
5	Isobyakangelicol	$C_{17}H_{16}O_6$	$[M+H]^+$	−0.5	122.79	317.101 9	188.095 7, 203.030 8, 218.018 9, 233.041 9, 218.018 9	B
6	12-Hydroxystearic acid	$C_{18}H_{36}O_3$	$[M-H]^-$	0.9	88.69	299.300 5	257.265 0, 256.261 0	B
7	Cryptochlorogenic acid	$C_{16}H_{18}O_9$	$[M-H]^-$	0.8	48.71	353.087 8	191.057 3, 179.036 8, 173.047 0, 135.046 5, 353.089 3	C
8	Ferulic acid	$C_{10}H_{10}O_4$	$[M-H]^-$	0.3	51.56	193.050 6	134.040 8, 149.062 4, 178.025 3, 193.050 9	C
9	8-Hydroxypsoralen	$C_{11}H_6O_4$	$[M+H]^+$	−1.4	72.06	203.033 8	203.032 2, 175.037 3, 147.042 5, 129.032 8	C
10	Senkyunolide J	$C_{12}H_{18}O_4$	$[M+H]^+$	−2.5	61.89	227.127 7	227.127 8, 191.106 2, 153.053 4, 217.106 6	C
11	Senkyunolide I	$C_{12}H_{16}O_4$	$[M+H]^+$	−0.5	66.64	225.094 0	207.100 0, 189.089 8, 179.106 1, 161.095 1, 247.093 9	C
12	Senkyunolide H	$C_{12}H_{16}O_4$	$[M+H]^+$	−3.5	69.54	225.112 1	207.099 9, 189.089 4, 165.052 9	C
13	Ligustilide	$C_{12}H_{14}O_2$	$[M+H]^+$	−1.1	104.23	191.106 6	119.084 1, 191.103 6, 173.095 9, 145.096 9, 115.055 5	C
14	Butylidenephthalide	$C_{12}H_{12}O_2$	$[M+H]^+$	−1.5	104.81	189.091 0	171.079 7, 161.091 8, 153.066 4, 128.060 7, 133.027 1	C
15	Senkyunolide F	$C_{12}H_{14}O_3$	$[M-H]^-$	1.8	122.69	205.087 0	186.932 0, 161.097 7, 205.086 8, 106.043 8	C
16	Senkyunolide A	$C_{12}H_{16}O_2$	$[M+H]^+$	0.3	125.98	193.104 2	179.070 9, 165.053 6, 163.075 4, 147.043 2, 187.074 6	C
17	Ligustrazine	$C_8H_{12}N_2$	$[M+H]^+$	−2.0	13.07	137.107 0	103.059 1, 122.063 4	C
18	Tectochrysin	$C_{16}H_{12}O_4$	$[M+H]^+$	−1.3	118.87	269.080 8	269.078 5, 237.052 7, 253.046 7, 118.040 0	F
19	Fraxidin	$C_{11}H_{10}O_5$	$[M+H]^+$	−3.3	57.61	223.060 1	162.029 5, 190.024 1, 223.057 8, 208.034 7, 193.009 5	F
20	Prim-*O*-glucosylcimifugin*	$C_{22}H_{28}O_{11}$	$[M+H]^+$	−1.9	48.50	469.170 4	261.108 6, 290.111 6, 307.113 4, 307.114 5, 235.057 5	F
21	Cimifugin*	$C_{16}H_{18}O_6$	$[M+H]^+$	−0.9	57.06	307.117 6	221.041 5, 235.057 0, 259.056 9, 289.104 2, 177.052 2	F
22	5-*O*-Methylvisammioside	$C_{22}H_{28}O_{10}$	$[M+H]^+$	−1.3	59.63	453.175 5	203.067 0, 219.061 5, 243.061 3, 245.113 6, 273.108 0	F
23	5-*O*-Methylvisamminol	$C_{16}H_{18}O_5$	$[M+H]^+$	−0.7	73.29	291.122 7	205.047 9, 219.063 2, 243.062 8, 273.110 8, 189.052 5	F
24	Sec-*O*-Glucosylhamaudol	$C_{21}H_{26}O_{10}$	$[M+H]^+$	−1.7	77.85	439.159 8	205.047 5, 259.094 2, 277.104 6, 217.047 0, 439.157 2	F
25	Vicenin-2	$C_{27}H_{30}O_{15}$	$[M-H]^-$	0.2	51.64	593.151 1	353.065 3, 383.078 3, 473.110 6, 593.152 0, 503.132 9	G
26	Vicenin-1	$C_{26}H_{28}O_{14}$	$[M-H]^-$	1.5	54.87	563.140 6	353.066 9, 383.079 5, 443.096 6, 473.110 9, 503.117 2	G
27	Schaftoside	$C_{26}H_{28}O_{14}$	$[M-H]^-$	0.8	55.87	563.140 6	353.065 5, 383.076 6, 443.097 0, 473.109 6, 503.117 5,	G
28	Isoschaftoside	$C_{26}H_{28}O_{14}$	$[M-H]^-$	0.7	56.28	563.140 6	353.067 5, 383.076 9, 443.099 1, 473.109 5	G
29	Liquiritigenin-7,4-diglucoside	$C_{27}H_{32}O_{14}$	$[M+H]^+$	−3.7	57.14	581.186 4	137.023 4, 257.079 2, 419.131 6, 581.185 4	G
30	Neoliquiritin	$C_{21}H_{22}O_9$	$[M+H]^+$	−1.1	58.66	419.133 6	91.057 5, 137.021 9, 257.078 5	G
31	Liquiritin*	$C_{21}H_{22}O_9$	$[M+H]^+$	−1.7	59.31	419.133 6	119.049 0, 137.022 0, 147.042 6, 211.073 2, 239.068 7	G
32	Isoviolanthin	$C_{27}H_{30}O_{14}$	$[M-H]^-$	3.3	60.57	577.156 2	353.066 9, 383.078 1, 457.114 9, 577.158 7	G
33	Liquiritin apioside	$C_{26}H_{30}O_{13}$	$[M-H]^-$	2.0	61.08	549.161 3	255.064 7, 429.106 0, 549.164 2, 417.120 1	G

Continued

No.	Identification	Formula	Ion adduction	Error /10^{-6}	t_R /min	Measured mass (*m/z*)	Fragment ion (*m/z*)	Source
34	Isoliquiritigenin	$C_{15}H_{12}O_4$	$[M+H]^+$	−1.0	61.63	257.080 8	137.021 8, 147.043 5, 242.058 4, 257.078 3	G
35	Liquiritigenin	$C_{15}H_{12}O_4$	$[M+H]^+$	−1.1	62.30	257.080 8	119.049 1, 137.022 3, 147.042 4, 165.067 9, 211.073 3	G
36	Isoliquiritin apioside*	$C_{26}H_{30}O_{13}$	$[M-H]^-$	2.2	64.18	549.161 3	255.066 5, 417.120 1, 429.117 6, 135.009 9	G
37	Choerospondin	$C_{21}H_{22}O_{10}$	$[M-H]^-$	1.2	70.92	433.114 0	271.026 4, 255.030 2, 151.004 6, 119.948 4	G
38	5,7,4′-Trihydroxyisoflavanone 7-*O*-glucoside	$C_{21}H_{22}O_{10}$	$[M-H]^-$	0.6	74.58	433.114 0	107.013 3, 151.004 5, 271.062 0, 433.114 3, 119.051 2	G
39	6″-*O*-Acetylliquiritin	$C_{23}H_{24}O_{10}$	$[M-H]^-$	−2.3	80.88	459.129 6	459.131 4, 255.066 9, 135.009 5, 119.051 2	G
40	Liguiritigenin-7-*O*-*D*-apiosyl-4′-*O*-*D*-glucoside	$C_{26}H_{30}O_{13}$	$[M-H]^-$	1.0	82.93	549.161 3	255.066 9, 429.104 6, 135.009 4, 119.050 9	G
41	Licorice glycoside C1	$C_{36}H_{38}O_{16}$	$[M-H]^-$	3.5	86.34	725.208 7	255.066 2, 531.152 4, 549.164 0	G
42	Licorice glycoside D2	$C_{35}H_{36}O_{15}$	$[M-H]^-$	2.9	87.90	695.198 1	255.066 7, 531.151 4, 549.161 6, 399.108 3, 145.029 7	G
43	Licochalcone B	$C_{16}H_{14}O_5$	$[M-H]^-$	0.7	92.59	285.076 8	150.033 0, 270.054 1, 149.025 4, 177.019 8	G
44	Licorice glycoside A	$C_{36}H_{38}O_{16}$	$[M-H]^-$	4.6	99.22	725.208 7	549.178 3, 531.154 9, 255.046 4, 193.051 4	G
45	1,3-5-Ethoxy-benzenedicarboxylicacid	$C_{10}H_{10}O_5$	$[M-H]^-$	2.9	47.02	209.045 5	59.018 6, 93.036 7, 165.056 6, 119.050 7, 209.046 7	G
46	Licoricesaponin A3	$C_{48}H_{72}O_{21}$	$[M-H]^-$	4.2	98.61	984.449 3	821.397 7, 645.370 4, 351.058 3, 759.409 0	G
47	Uralsaponin F	$C_{44}H_{64}O_{19}$	$[M-H]^-$	3.4	103.37	895.396 9	351.057 1, 895.401 6	G
48	Licoricesaponin G2	$C_{42}H_{62}O_{17}$	$[M+H]^+$	−2.1	118.16	839.405 9	451.318 2, 469.327 2, 487.338 2, 663.370 0	G
49	Licoricesaponin H2	$C_{42}H_{62}O_{16}$	$[M+H]^+$	−3.8	121.84	823.411 0	453.332 2, 471.343 2, 647.373 5, 823.407 9	G
50	Melliferone	$C_{30}H_{44}O_3$	$[M+H]^+$	−3.0	123.21	453.336 3	407.337 0, 435.334 2, 357.243 6, 303.198 8, 389.317 4	G
51	Ammonium glycyrrhizate*	$C_{42}H_{65}NO_{16}$	$[M+H]^+$	−3.8	124.53	840.434 7	453.322 5, 471.342 4, 647.375 2, 823.407 1	G
52	Cassiaside B	$C_{26}H_{30}O_{14}$	$[M-H]^-$	1.5	72.84	565.156 2	271.061 9, 565.157 2	G
53	Licocoumarone	$C_{20}H_{20}O_5$	$[M+H]^+$	0.6	127.37	341.138 3	149.058 3, 285.073 7, 341.136 9, 300.097 3, 285.073 7	G
54	Gallic acid*	$C_7H_6O_5$	$[M-H]^-$	2.9	24.23	169.014 2	125.025 3, 169.014 7, 97.031 1, 79.021 6	H1
55	Vitamin C	$C_6H_8O_6$	$[M-H]^-$	0.6	14.18	175.024 8	69.037 5, 87.046 9, 113.125 5, 157.014 6, 175.022 3	H1
56	Pratensein-7-*O*-glucoside	$C_{22}H_{22}O_{11}$	$[M-H]^-$	2.4	55.52	461.108 9	461.109 3, 299.056 1, 284.032 8	H2
57	Calycosin	$C_{16}H_{12}O_5$	$[M+H]^+$	−1.6	57.12	285.075 7	137.022 0, 197.056 0, 213.051 7, 225.050 8, 253.046 7	H2
58	Calycosin 7-*O*-glucoside	$C_{22}H_{22}O_{10}$	$[M-H]^-$	1.6	72.35	445.114 0	239.076 1, 268.039 5, 283.062 3, 491.121 3	H2
59	Calycosin-7-*O*-*β*-*D*-(6″-acetyl)-glucoside	$C_{24}H_{24}O_{11}$	$[M+H]^+$	−2.6	74.22	489.139 1	285.073 7, 489.138 4, 175.037 4, 229.083 5	H2
60	3′-Hydroxy-4′-methoxy isoflavone	$C_{16}H_{12}O_4$	$[M-H]^-$	2.5	75.14	267.066 2	251.035 6, 223.041 2, 195.046 6, 132.021 1, 165.057 5	H2
61	Isomucronulatol	$C_{17}H_{18}O_5$	$[M+H]^+$	−1.6	80.48	303.122 7	303.146 3, 167.067 8, 133.069 3, 123.043 7, 161.056 7	H2
62	4′,6-Dimethoxy-7-hydroxyisoflavone	$C_{17}H_{20}O_5$	$[M+H]^+$	0.4	81.03	305.091 4	203.032 7, 175.037 8, 160.014 5, 188.008 6	H2

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No.	Identification	Formula	Ion adduction	Error /10^{-6}	t_R /min	Measured mass (*m/z*)	Fragment ion (*m/z*)	Source
63	Astragaloside IV	$C_{41}H_{68}O_{14}$	$[M+COOH]^-$	4.8	103.39	830.458 0	783.457 7, 829.462 8, 784.461 2	H2
64	Astragaloside II	$C_{43}H_{70}O_{15}$	$[M+H]^+$	−0.6	112.78	827.478 7	827.474 5, 809.457 8, 647.410 1, 473.358 3, 455.349 3	H2
65	Isoastragaloside I	$C_{45}H_{72}O_{16}$	$[M+H]^+$	−1.4	124.00	870.471 2	653.399 5, 437.339 2, 419.328 8, 851.480 1, 689.421 3	H2
66	Raffinose	$C_{18}H_{32}O_{16}$	$[M-H]^-$	−2.3	5.39	503.161 7	503.162 1, 161.046 9, 179.055 8, 89.026 3, 101.025 6	H2
67	Homoorientin	$C_{21}H_{20}O_{11}$	$[M+H]^+$	−2.6	56.41	449.107 8	329.063 4, 431.096 0, 449.105 8, 413.096 0, 353.064 6	X1
68	Hesperidin	$C_{28}H_{34}O_{15}$	$[M-H]^-$	2.6	70.79	609.182 4	301.072 0, 343.084 3, 269.045 0, 609.184 4, 383.115 1	X1
69	Danshensu	$C_9H_{10}O_5$	$[M-H]^-$	3.1	29.52	197.047 7	123.047 9, 135.047 2, 179.036 8, 197.047 7	X1
70	Salviaflaside	$C_{24}H_{26}O_{13}$	$[M-H]^-$	1.2	67.54	521.130 0	161.025 0, 359.077 4, 179.035 6, 323.077 0, 135.045 6	X1
71	Rosmarinic acid*	$C_{18}H_{16}O_8$	$[M-H]^-$	1.4	79.31	359.077 2	135.046 0, 161.025 0, 179.046 5, 197.046 5, 359.078 9	X1
72	Gluconic acid	$C_6H_{12}O_7$	$[M-H]^-$	0.4	4.43	195.051 0	59.018 3, 75.012 1, 195.052 2, 129.021 6, 159.031 9	X1
73	Malic acid	$C_4H_6O_5$	$[M-H]^-$	2.9	6.54	133.014 2	71.017 0, 72.996 2, 115.004 8, 133.015 1, 59.017 3	X1
74	Eudesmin	$C_{22}H_{26}O_6$	$[M+H]^+$	−1.4	70.75	387.180 2	369.168 1, 351.157 8, 339.158 0, 231.099 4, 189.089 3	X2
75	Denudatone	$C_{22}H_{26}O_6$	$[M+H]^+$	−0.3	80.28	387.180 2	369.168 5, 351.157 9, 339.158 7, 231.099 2, 189.089 5	X2
76	Magnone A	$C_{22}H_{26}O_7$	$[M+H]^+$	−0.8	93.46	403.175 1	385.162 4, 367.154 1, 265.105 7, 247.094 9, 217.084 3	X2
77	Magnone B	$C_{23}H_{28}O_8$	$[M+H]^+$	−0.7	101.68	433.185 6	415.173 0, 397.162 8, 265.105 3, 247.097 4, 217.083 2	X2
78	*epi*-Magnolin A	$C_{23}H_{28}O_7$	$[M+H]^+$	−2.5	117.25	417.190 7	399.178 8, 381.167 6, 369.167 1, 329.136 7, 261.110 3	X2
79	Yangambin	$C_{24}H_{30}O_8$	$[M+H]^+$	−1.8	119.86	447.201 3	429.188 3, 411.177 7, 380.159 4, 358.138 7, 231.099 1	X2
80	Magnolin*	$C_{23}H_{28}O_7$	$[M+H]^+$	−1.7	122.36	417.190 7	439.176 0, 254.092 5, 151.073 4, 108.995 0, 381.167 5	X2
81	Senkyunolide C	$C_{12}H_{12}O_3$	$[M-H]^-$	1.5	127.62	203.071 3	203.072 4, 173.025 0, 159.893 1, 145.030 2, 116.928 2	X2
82	Taxifolin-7-*O*-glucoside	$C_{21}H_{22}O_{12}$	$[M-H]^-$	0.4	55.43	465.103 8	303.051 4, 465.104 9, 285.039 1, 135.046 4, 166.999 1	Y
83	Eriodictyol-7-*O*-glucoside	$C_{21}H_{22}O_{11}$	$[M-H]^-$	0.3	62.52	449.108 9	135.045 6, 151.004 2, 175.004 7, 287.057 3, 449.110 2	Y
84	Isovitexin	$C_{21}H_{20}O_{10}$	$[M-H]^-$	2.2	62.76	431.098 3	431.099 4, 341.066 9, 311.057 5, 283.064 4	Y
85	Luteolin-7-*O*-rutinoside	$C_{27}H_{30}O_{16}$	$[M-H]^-$	2.2	65.29	609.151 1	285.041 3, 593.153 5	Y
86	Kaempferol-7-*O*-glucoside	$C_{21}H_{20}O_{11}$	$[M-H]^-$	0.9	67.62	447.093 2	447.093 7, 285.040 6, 284.032 7, 327.051 4	Y
87	Apigenin-7-*O*-rutinoside	$C_{27}H_{30}O_{14}$	$[M-H]^-$	1.4	71.54	577.156 2	269.046 3, 577.158 1, 311.064 7, 397.059 7	Y
88	5,3′,4′-Tetrahydroxy-dihydroflavone-7-*O*-glucoside	$C_{21}H_{22}O_{11}$	$[M-H]^-$	−0.6	73.36	449.108 9	287.057 4, 151.004 7, 107.015 3	Y
89	Diosmetin-7-*O*-glucoside	$C_{22}H_{22}O_{11}$	$[M-H]^-$	1.0	74.26	461.108 9	461.110 2, 299.057 6, 284.035 9	Y
90	Hesperetin 7-*O*-glucuronide	$C_{22}H_{22}O_{12}$	$[M-H]^-$	3.6	77.73	477.103 8	301.073 9, 113.026 7, 151.002 9, 175.024 9, 477.104 2	Y
91	Quercitrin	$C_{21}H_{20}O_{11}$	$[M-H]^-$	0.9	77.91	447.093 2	300.027 8, 301.036 2, 447.093 2, 271.062 2, 151.003 6	Y

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92	Hesperetin	$C_{16}H_{14}O_6$	$[M-H]^-$	−0.4	79.15	301.071 7	301.072 8, 286.053 0, 164.929 1, 151.039 2	Y
93	Linarin*	$C_{28}H_{32}O_{14}$	$[M+H]^+$	−2.3	80.26	593.186 4	285.072 8, 447.125 3, 593.183 4	Y
94	Diosmetin 7-*O*-*β*-*D*-glucuronide	$C_{22}H_{20}O_{12}$	$[M-H]^-$	1.6	80.42	475.088 2	475.089 6, 299.056 3, 284.032 7, 113.026 1	Y
95	Apigenin 7-glucuronide	$C_{21}H_{18}O_{11}$	$[M-H]^-$	−0.8	81.05	445.077 4	269.046 2, 113.025 5, 175.026 0, 308.917 0, 194.906 5	Y
96	Luteolin-7-*O*-6″-acetylglucoside	$C_{23}H_{22}O_{12}$	$[M-H]^-$	2.1	84.19	489.103 8	285.040 9, 489.104 6, 284.034 5	Y
97	Caffeic acid-glucoside	$C_{15}H_{18}O_9$	$[M-H]^-$	2.0	44.04	341.087 8	281.068 0, 251.055 0, 221.047 4, 179.035 6, 161.026 3	Y
98	1-*O*-Caffeoylquinic acid	$C_{16}H_{18}O_9$	$[M+H]^+$	−1.0	46.51	355.102 3	89.039 6, 107.048 6, 117.032 4, 135.043 0, 145.027 4	Y
99	*p*-Coumaroylquinic acid	$C_{16}H_{18}O_8$	$[M-H]^-$	0.6	56.02	337.092 8	93.036 8, 191.035 8, 147.046 7, 191.035 8, 163.041 0	Y
100	Methyl caffeate	$C_{10}H_{10}O_4$	$[M-H]^-$	3.4	66.45	193.050 6	133.030 4, 178.028 0, 193.051 2, 134.038 2, 149.062 1	Y
101	1,3-Dicaffeoylquinic acid	$C_{25}H_{24}O_{12}$	$[M-H]^-$	0.6	76.56	515.119 5	515.119 9, 353.087 6, 191.056 5, 179.035 5, 173.046 4	Y
102	1,5-Dicaffeoylquinic acid*	$C_{25}H_{24}O_{12}$	$[M-H]^-$	1.0	78.14	515.119 5	515.120 8, 353.088 6, 191.056 7, 179.035 5, 173.047 5	Y
103	4,5-Dicaffeoylquinic acid	$C_{25}H_{24}O_{12}$	$[M-H]^-$	0.5	82.19	515.119 5	515.122 3, 353.089 8, 191.057 2, 179.036 2, 173.046 9	Y
104	Quinic acid	$C_7H_{12}O_6$	$[M-H]^-$	1.0	5.17	191.056 1	191.055 6, 173.045 7, 127.041 5, 93.036 5, 87.011 4	Y
105	Benzoic acid	$C_7H_6O_2$	$[M-H]^-$	4.2	67.17	121.029 5	77.042 4, 121.030 9, 91.023 1, 92.028 7	Y
106	Methylparaben	$C_8H_8O_3$	$[M-H]^-$	4.8	43.69	151.040 0	108.023 5, 136.017 8, 151.041 0, 109.031 2	Y
107	Sucrose	$C_{12}H_{22}O_{11}$	$[M+COOH]^-$	0.3	4.64	387.113 3	387.113 4, 341.107 3, 179.056 0	B, C, H2, X1
108	5-Hydroxyl-8-methoxypsoralen	$C_{12}H_8O_5$	$[M+H]^+$	−0.7	80.34	233.044 4	218.018 5, 190.023 8, 162.029 2, 134.035 1, 106.040 7	B, F
109	Byakangelicin	$C_{17}H_{18}O_7$	$[M+H]^+$	−0.8	81.00	335.094 4	162.029 3, 238.993 5, 203.031 5, 254.016 9	B, F
110	Scopoletin	$C_{10}H_8O_4$	$[M+H]^+$	−1.9	57.77	193.049 5	178.024 3, 161.023 6, 133.027 1, 137.058 0, 149.025 1	B, F, G
111	Nodakenin	$C_{20}H_{24}O_9$	$[M+H]^+$	−2.9	57.86	409.149 3	247.095 0, 229.083 7, 201.087 4, 187.036 6, 175.037 4	B, F, H2
112	Caffeic acid	$C_9H_8O_4$	$[M-H]^-$	3.1	52.95	179.034 9	89.040 9, 107.052 4, 134.038 3, 135.045 7, 179.035 6	C, F, H2, X1, X2
113	Myristicin	$C_{11}H_{12}O_3$	$[M+H]^+$	−2.2	38.26	193.085 9	193.083 9, 161.058 3, 133.065 3, 131.047 6, 115.054 0	C, F, X1, X2
114	Vanillic acid	$C_8H_8O_4$	$[M-H]^-$	3.4	28.37	167.034 9	108.023 6, 167.035 8, 123.046 5, 137.025 8	C, F, X1, X2, Y
115	Cosmosiin	$C_{21}H_{20}O_{10}$	$[M-H]^-$	0.4	74.75	431.098 3	269.046 3, 431.100 9, 268.039 1, 269.036 4, 311.059 3	C, H2, Y
116	3,5-Dicaffeoylquinic acid	$C_{25}H_{24}O_{12}$	$[M-H]^-$	3.0	59.85	515.119 5	353.088 4, 191.056 9, 179.036 0, 135.046 2	C, H2, Y
117	Citric Acid	$C_6H_8O_7$	$[M-H]^-$	0.8	11.51	191.019 7	85.031 7, 87.011 0, 111.009 9, 129.024 2, 173.009 0	C, X1
118	Sinapic acid	$C_{11}H_{12}O_5$	$[M-H]^-$	2.3	61.51	223.061 2	223.061 3, 163.041 2, 133.065 3, 91.057 3, 117.072 0	C, X1, X2, Y
119	Chlorogenic acid	$C_{16}H_{18}O_9$	$[M-H]^-$	0.2	39.84	353.087 8	161.041 5, 135.046 1, 179.035 5, 191.056 3, 353.087 7	C, Y, H2, X1
120	Neochlorogenic acid	$C_{16}H_{18}O_9$	$[M+H]^+$	−2.2	37.75	355.101 8	89.039 0, 117.033 1, 135.042 9, 145.026 8, 163.037 5	C, Y

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No.	Identification	Formula	Ion adduction	Error /10^{-6}	t_R /min	Measured mass (*m/z*)	Fragment ion (*m/z*)	Source
121	Acteoside	$C_{29}H_{36}O_{15}$	$[M-H]^-$	0.5	69.05	623.198 1	161.025 6, 461.168 1, 623.199 8	F, G
122	Umbelliferone	$C_9H_6O_3$	$[M+H]^+$	−2.4	46.13	163.038 7	135.042 4, 117.032 9, 89.039 0, 92.026 7, 107.049 8	F, X1, X2, Y
123	Baicalin	$C_{21}H_{18}O_{11}$	$[M+H]^+$	−4.0	78.77	447.092 1	271.058 4, 447.089 6, 213.015 1, 228.037 7	F, Y
124	3,4-Dicaffeoylquinic acid	$C_{25}H_{24}O_{12}$	$[M-H]^-$	0.6	78.60	515.119 5	515.118 4, 353.099 7, 191.057 1, 179.036 1	F, Y
125	Rutin*	$C_{27}H_{30}O_{16}$	$[M-H]^-$	1.2	67.51	609.146 1	300.028 5, 301.035 3, 609.174 7, 463.088 4, 271.063 3	G, H1, H2, X1
126	Formononetin	$C_{16}H_{12}O_4$	$[M+H]^+$	−1.0	73.02	269.080 8	118.040 4, 197.057 3, 226.059 2, 213.088 4, 237.053 7	G, H2
127	Ononin	$C_{22}H_{22}O_9$	$[M+H]^+$	−1.4	73.05	431.133 6	254.055 1, 269.078 5, 270.081 3, 431.132 7, 432.136 2	G, H2
128	Naringenin-7-*O*-glucoside	$C_{21}H_{22}O_{10}$	$[M-H]^-$	0.5	65.90	433.134 0	120.056 3, 119.052 5, 151.004 2, 271.061 7, 433.113 8	G, Y
129	Kaempferol-3-rutinoside	$C_{27}H_{30}O_{15}$	$[M-H]^-$	3.4	72.74	593.151 1	593.154 6, 285.040 7, 284.034 2	G, Y
130	Chrysoeriol	$C_{16}H_{12}O_6$	$[M-H]^-$	1.9	84.95	299.056 1	227.035 5, 240.043 3, 299.056 7, 255.065 7	G, Y
131	Physcion	$C_{16}H_{12}O_5$	$[M+H]^+$	−0.9	92.09	285.075 7	242.055 8, 270.050 4, 285.073 9, 196.048 8, 197.057 8	G, Y
132	Ellagic acid*	$C_{14}H_6O_8$	$[M-H]^-$	1.2	69.75	300.998 9	229.992 0, 257.011 1, 300.999 5	H1, Y
133	Protocatechuic acid	$C_7H_6O_4$	$[M-H]^-$	3.7	35.00	153.019 3	108.023 4, 109.031 5, 153.020 5	X1, X2
134	*p*-Coumaric acid	$C_9H_8O_3$	$[M-H]^-$	3.6	66.18	163.040 0	93.036 7, 117.035 1, 119.051 8, 163.041 3, 91.056 9	X1, X2, Y
135	Cynaroside	$C_{21}H_{20}O_{11}$	$[M-H]^-$	0.5	76.21	447.093 2	284.032 8, 285.041 1, 327.050 9, 255.030 9, 227.053 5	X1, Y
136	Luteolin*	$C_{15}H_{10}O_6$	$[M-H]^-$	0.1	103.92	285.040 6	285.040 9, 217.050 0, 199.040 1, 175.039 8, 151.004 6	X1, Y
137	Methyl vanillate	$C_9H_{10}O_4$	$[M-H]^-$	1.6	36.41	181.050 6	107.052 1, 135.046 5, 119.051 7, 163.040 8, 181.051 7	Y, X2

3 蛋白−蛋白相互作用 (protein-protein interaction, PPI) 及作用通路分析

将63个关键靶点导入String数据库, 构建PPI网络图, 利用Cytoscape软件对分析结果进行可视化 (图1)。隐藏孤立靶点后该网络中共有62个节点, 601条边, 节点颜色和大小与度值有关。其中根据度值排名靠前的核心靶点有肿瘤坏死因子 (tumor necrosis factor, TNF)、白细胞介素1B (interleukin 1B, IL1B)、蛋白激酶B (protein kinase B, AKT1)、血管内皮生长因子A (vascular endothelial growth factor A, VEGFA) 和信号传导及转录激活蛋白-3 (signal transducer and activator of transcription 3, STAT3)。利用Cluego插件对关键靶点进行通路富集分析, 以$P < 0.01$为筛选条件, 共富集到76条通路, 结果如图2所示, 图中每个圆点代表一条通路, P值越小圆点越大, 由图可以得知关键靶点显著富集在晚期糖基化产物−晚期糖基化终末产物受体 (advanced glycosylation end products-receptor of AGEs, AGE-RAGE)、TNF、核转录因子κB (nuclear factor kappa B, NF-κB) 和环磷酸鸟苷−蛋白激酶G (cyclic guanosine monophosphate/protein kinase G, cGMP-PKG) 等信号通路。

4 “中药−成分−靶点”网络图的构建

借助Cytoscape软件构建“中药−成分−靶点”网络图并进行拓扑学分析, 结果如图3所示。度值 (degree) 是网络图分析中重要的拓扑学参数, 其值的大小与作用能力成正相关。各成分的度值结果见表2, 排名前10位的活性成分分别为: 咖啡酸、洋川芎内酯F、迷迭香酸、藁本内酯、升麻素苷、蒙花苷、木兰脂素、木犀草素、洋川芎内酯I及没食子酸, 这些成分可能是香菊制剂治疗鼻炎、鼻窦炎的重要活性成分。

5 分子对接验证

对香菊制剂治疗鼻炎、鼻窦炎的度值靠前的10个重要活性成分与5个核心靶点进行分子对接验证。当结合能 < 0 kcal·mol^{-1}时, 配体与受体可自发产生相应

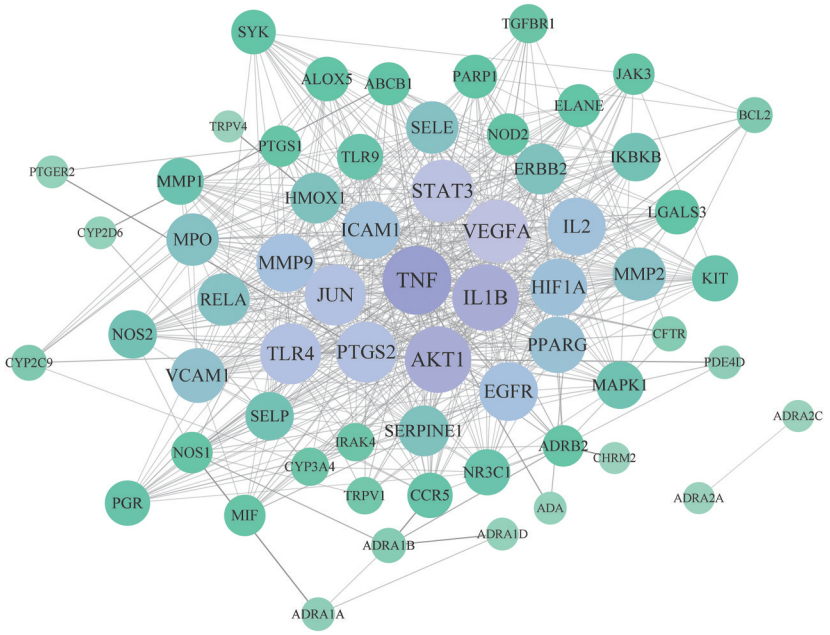

Figure 1 Protein-protein interaction (PPI) network interaction of key targets of Xiangju preparations

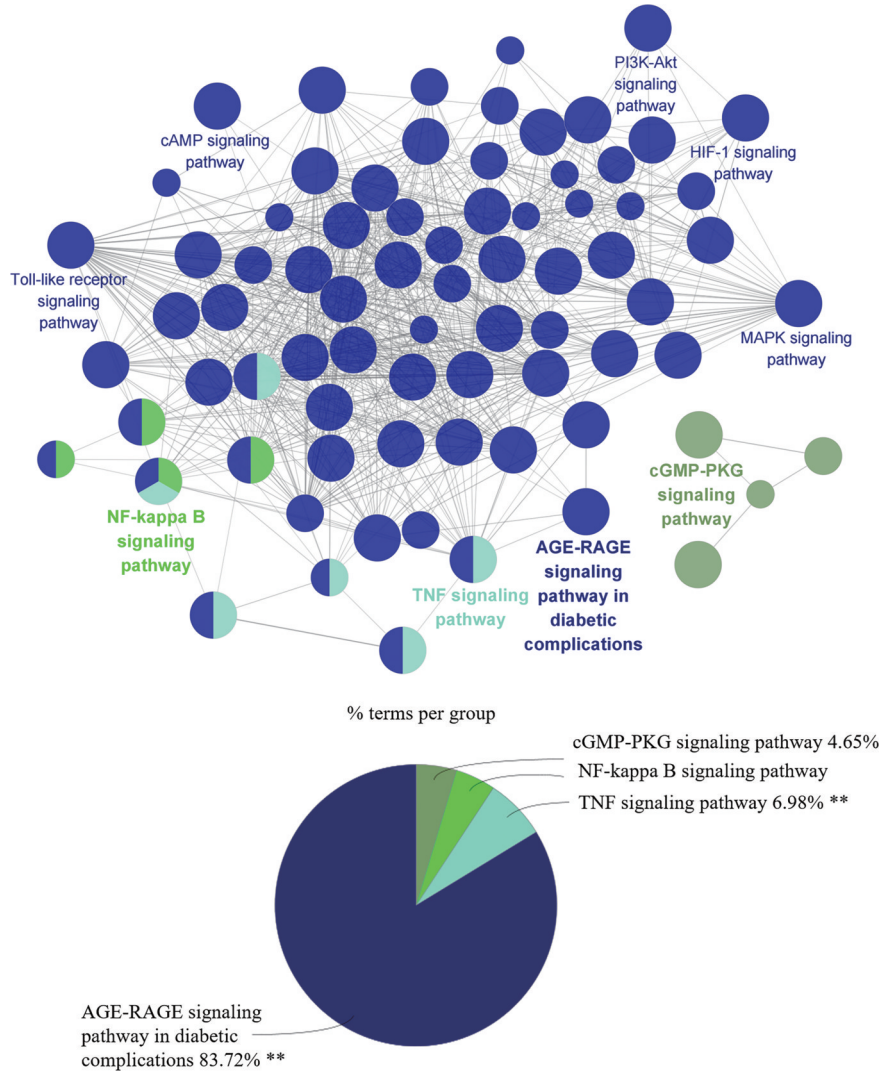

Figure 2 Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis

Table 2 The information of potential active ingredients

PubChem CID	Ingredient	Source	Degree
5280448	Calycosin	H2	7
503737	Liquiritin	G	3
114829	Liquiritigenin	G	11
5280378	Formononetin	H2, G	8
64982	Baicalin	F, Y	8
72281	Hesperetin	Y	9
5317025	Linarin	Y	14
5280666	Chrysoeriol	G, Y	3
5318999	Licochalcone B	G	9
5280445	Luteolin	X1, Y	13
73191	Nodakenin	H2, F, B	4
234823	Eudesmin	X2	3
148619	Isogosferol	B	2
6474640	1,3-Dicaffeoylquinic acid	Y	8
5281855	Ellagic acid	H1, Y	7
441970	5-*O*-Methylvisamminol	F	7
503731	Licocoumarone	G	5
445858	Ferulic acid	C	11
5281792	Rosmarinic acid	X1	16
169234	Magnolin	X2	13
13943297	Astragaloside IV	H2	9
62074	Ammonium glycyrrhizate	G	5
14034912	prim-*O*-Glucosylcimifugin	F	15
21670038	5-*O*-Methylvisammioside	F	3
11521428	Senkyunolide I	C	13
10478277	sec-*O*-Glucosylhamaudol	F	5
5281954	Tectochrysin	F	9
370	Gallic acid	H1	12
44257307	Pratensein-7-*O*-glucoside	H2	3
9867450	Magnone B	X2	6
5280459	Quercitrin	Y	7
5280460	Scopoletin	B, F, G	3
8468	Vanillic acid	C, F, Y, X1, X2	9
5280704	Cosmosiin	C, Y, H2	8
92794	Naringenin-7-*O*-glucoside	Y, G	4
637542	*p*-Coumaric acid	Y, X1, X2	10
17536	Oxypeucedanin hydrate	B	6
160544	Oxypeucedanin	B	6
10211	Byakangelicin	B, F	3
689043	Caffeic acid	C, F, H2, X1, X2	26
6438919	Salviaflaside	X1	4
9798666	Cryptochlorogenic acid	C	5
5280633	Neochlorogenic acid	C, Y	6
1794427	Chlorogenic acid	C, Y, H2, X1	8
51666248	Neoliquiritin	G	3
14187172	Licoricesaponin A3	G	5
14891565	Licoricesaponin G2	G	11
14837471	Licoricesaponin H2	G	3
13996693	Astragaloside II	H2	5
10076238	Liquiritin apioside	G	10
5280805	Rutin	G, H1, H2, X1	9
24121290	Senkyunolide J	C	3
5319022	Ligustilide	C	15
642376	Butylidenephthalide	C	6
11241196	Senkyunolide F	C	18
3085257	Senkyunolide A	C	1
638278	Isoliquiritigenin	G	1
5318267	Calycosin 7-*O*-glucoside	H2	3
10621	Hesperidin	X1	1

的作用, 结合能 < 5 kcal·mol^{-1}时表明其结合性较好, 结合能越小, 配体与受体的结合越稳定。如表3所示, 10个活性成分均可自发地与5种核心蛋白结合, 从而发挥生物活性。

讨论

在本研究中, 应用HPLC-QTOF/MS技术, 初步鉴定了覆盖香菊制剂中9种中药材的137个化学成分。随后针对其中59个潜在活性成分进行系统的网络药理学研究, 从整体生物效应网络角度系统探讨了香菊制剂治疗鼻炎、鼻窦炎的药效物质基础及其作用机制。

本研究利用网络药理学的方法探讨了香菊制剂治疗鼻炎、鼻窦炎的作用机制。通过对香菊制剂治疗鼻炎、鼻窦炎的关键靶点进行PPI网络分析可以得知, TNF、IL1B、AKT1、VEGFA及STAT3等靶点在整个网络中具有较好的互作关系。这些核心靶点在鼻炎、鼻窦炎的发生发展过程中扮演着重要的角色。其中TNF、IL1B与STAT3均与炎症反应有关, 可通过促进炎症细胞浸润、活化并释放炎症介质参与鼻部炎症的发生[28]。AKT1和VEGFA则在细胞凋亡、组织重塑和呼吸系统变应性炎症中起着关键作用[29]。通路富集分析结果表明, 这些关键靶点主要富集在AGE-RAGE、TNF、NF-κB及cGMP-PKG等信号通路。据报道, AGE-RAGE信号通路与鼻部炎症疾病具有很强的相关性, 受体RAGE的表达能够反映疾病部位炎症发展程度, 与AGEs结合后可激活丝裂原活化蛋白激酶(mitogen-activated protein kinase, MAPK)和NF-κB信号通路, 促进炎症细胞因子如TNF和IL1B的表达, 进而加重鼻窦黏膜形态病变和气道重塑, 导致鼻部炎症的发生[30]。此外, 本研究还富集到了TNF、cGMP-PKG、Toll样受体(toll-like receptor, TLR)及缺氧诱导因子1 (hypoxia inducible factor 1, HIF-1)等重要信号通路, 这些通路与免疫调节和炎症反应有关, 可指示鼻部炎症的严重程度, 在鼻炎、鼻窦炎相关文献[31,32]中均有报道。因此可以推断香菊制剂可通过调节多条炎症、免疫相关通路协同发挥治疗鼻炎、鼻窦炎的作用机制。

通过进一步构建“中药−成分−靶点”网络, 有效预测并筛选了10个潜在药效物质, 分别为咖啡酸、洋川芎内酯F、迷迭香酸、藁本内酯、升麻素苷、蒙花苷、木兰脂素、木犀草素、洋川芎内酯I及没食子酸。其中, 咖啡酸可通过抑制AGEs的形成阻断AGEs-RAGE的正反馈回路, 降低TNF-α和IL1β的表达, 起到抗炎作用[33]。蒙花苷、洋川芎内酯I、没食子酸、升麻素苷和木兰脂素等活性成分均能够抑制NF-κB信号通路, 下调

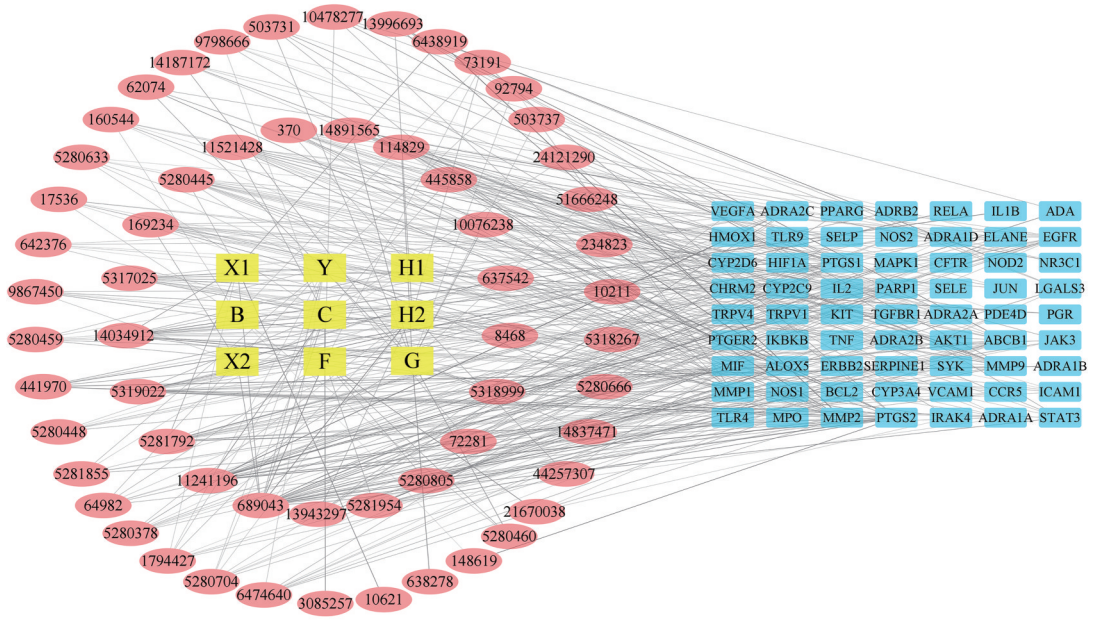

Figure 3 Network diagram of "drugs-ingredients-targets". Yellow: Drugs; Red: Ingredients; Blue: Targets

Table 3 The binding energy of active ingredients with key targets

PubChem CID	Component	Binding energy/kcal·mol^{-1}				
		TNF	IL1B	AKT1	VEGFA	STAT3
689043	Caffeic acid	−4.57	−5.88	−4.59	−4.97	−5.13
11241196	Senkyunolide F	−4.87	−6.16	−4.78	−5.03	−4.47
5281792	Rosmarinic acid	−4.27	−4.36	−3.9	−2.94	−4.36
5319022	Ligustilide	−3.85	−6.13	−5.81	−4.49	−5.18
14034912	prim-*O*-Glucosylcimifugin	−4.06	−5.64	−3.46	−2.92	−2.78
5317025	Linarin	−4.43	−4.44	−2.32	−2.65	−2.53
169234	Magnolin	−5.42	−6.44	−4.53	−5.2	−4.57
5280445	Luteolin	−3.49	−6.18	−4.73	−5.05	−4.83
11521428	Senkyunolide I	−4.05	−5.72	−4.37	−3.75	−4.3
370	Gallic acid	−3.82	−5.41	−3.8	−5.45	−4.31

促炎细胞因子 (TNF-α、IL1β) 和炎症介质的表达来改善鼻部炎症症状及细胞炎症损伤[34-38]。洋川芎内酯F和藁本内酯亦可作用于TNF信号通路发挥抗炎作用[39]。木犀草素可通过调节AKT1和VEGFA等蛋白水平, 发挥抗炎、促进免疫应答等作用[40]。迷迭香酸作为一种天然的抗氧化剂, 除了可通过增加活性氧的含量改善氧化应激反应及鼻黏膜损伤外[41], 还可通过调节STAT3蛋白的水平上调促凋亡因子的表达[42]。分子对接结果进一步验证了上述10个活性成分与核心靶点TNF、IL1B、AKT1、VEGFA、STAT3之间具有较好的结合性能, 说明了这10种化学成分是香菊制剂的主要药效物质。综上, 香菊制剂可通过多成分、多靶点及多通路来发挥治疗鼻炎、鼻窦炎的作用, 这也为香菊制剂的药效物质选择提供了依据。

本研究通过整合现代分析技术与生信手段, 构建了香菊制剂的“成分-靶点-通路”互作网络, 初步探讨了香菊制剂治疗鼻炎、鼻窦炎的作用机制及药效物质。研究结果为后续香菊制剂质量标准提升和作用机制深入研究提供了依据。

作者贡献: 李赛玉主要进行了本文的选题与设计、方法建立、实验数据处理、撰写文章等; 张译文对实验思路、文章撰写提供了指导; 杨盼盼在前期方法摸索及数据处理方面提供了帮助; 王欣然在样品制备及数据处理方面提供了帮助; 邢露文在前期文献调研提供了帮助; 李清对本文选题与设计、实验思路、方法建立及文章修改提供了宝贵意见。

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References

[1] Bachert C, Gevaert E. Advances in rhinitis and rhinosinusitis in 2015 [J]. J Allergy Clin Immunol, 2016, 138: 1277-1283.

[2] Staudacher AG, Peters AT, Kato A, et al. Use of endotypes, phenotypes, and inflammatory markers to guide treatment decisions in chronic rhinosinusitis [J]. Ann Allergy Asthma Immunol, 2020, 124: 318-325.

[3] Martin KC. Allergy, histamine and antihistamines [J]. Handb Exp Pharmacol, 2017, 241: 321-331.

[4] Wu YJ, Yang HR. Progress in the mechanism and application of antihistamines in allergic rhinitis [J]. Chin J Otorhinolaryngol Skull Base Surg (中国耳鼻咽喉颅底外科杂志), 2021, 27: 742-745.

[5] Liu JL. Effect of montelukast sodium combined with Xiangju tablets in the treatment of allergic rhinitis [J]. Clin Res Pract (临床医学研究与实践), 2018, 3: 132-133.

[6] Huang ZG, Bai HL, Ye JT, et al. The effect of Xiangju tablet on behavior of rats with allergic rhinitis and expression of aquaporin 3 in nasal mucosa [J]. Clin Misdiagn Misther (临床误诊误治), 2018, 31: 101-104.

[7] Sun JS, Li XH, Wang K, et al. Clinical study on Xiangju tablets combined with oxymetazoline hydrochloride spray in treatment of chronic sinusitis [J]. Drugs Clin (现代药物与临床), 2019, 34: 1422-1425.

[8] Wei YF, Zhang N, Lin X, et al. Release characteristics *in vitro* and pharmacokinetics of Da Chuanxiong Fang multiunit drug delivery system in rats [J]. Acta Pharm Sin (药学学报), 2011, 46: 1150-1155.

[9] Li YY, Zhang B, Yuan YF. Identification of components and metabolites of Saposhnikoviae Radix decoction in rats' plasma by UPLC-Q-TOF-MS [J]. J Pharm Pract (药学实践杂志), 2019, 37: 512-517.

[10] Zhao X, Su X, Liu C, et al. Simultaneous determination of chrysin and tectochrysin from *Alpinia oxyphylla* Fruits by UPLC-MS/MS and its application to a comparative pharmacokinetic study in normal and dementia rats [J]. Molecules, 2018, 23: 1702-1713.

[11] Hu KX, Ju HY, Han LZ, et al. Determination of gallic acid and ellagic acid content and HPLC fingerprint analysis in the *Platycaryastrobilacea* Sieb. et Zucc [J]. J Liaoning Univ Tradit Chin Med (辽宁中医药大学学报), 2020, 22: 56-59.

[12] Li SN, Liu CM, Zhang YC, et al. On-line coupling pressurised liquid extraction with two-dimensional counter current chromatography for isolation of natural acetylcholinesterase inhibitors from *Astragalus membranaceus* [J]. Phytochem Anal, 2021, 32: 640-653.

[13] Wang TS. Study on Fragmentation of seselinone and rumphiin by electrospray ion trap mass spectrometry [J]. J Hainan Norm Univ Nat Sci (海南师范大学学报 自然科学版), 2021, 34: 396-400.

[14] Ma ST, Zhang XY, Zhang N, et al. Quantitative determination of quercitrin levels in rat plasma using UHPLC-MS/MS and its application in a pharmacokinetic study after the oral administration of Polygoni Cuspidati Folium capsules [J]. Curr Pharm Biotechnol, 2022, 23: 457-465.

[15] Chang YX, Zhang QH, Li J, et al. Simultaneous determination of scopoletin, psoralen, bergapten, xanthotoxin, columbianetin acetate, imperatorin, osthole and isoimperatorin in rat plasma by LC-MS/MS for pharmacokinetic studies following oral administration of Radix Angelicae Pubescentis extract [J]. J Pharm Biomed Anal, 2013, 77: 71-75.

[16] Luo NC, Li ZH, Qian DW, et al. Simultaneous determination of bioactive components of Radix Angelicae Sinensis-Radix Paeoniae Alba herb couple in rat plasma and tissues by UPLC-MS/MS and its application to pharmacokinetics and tissue distribution [J]. J Chromatogr B Analyt Technol Biomed Life Sci, 2014, 963: 29-39.

[17] Chen ZX, Ying XX, Meng S, et al. LC determination of luteolin-7-*O*-*β*-*D*-glucoside and apigenin-7-*O*-*β*-*D*-glucoside in rat plasma after administration of *Humulus scandens* extract and its application to pharmacokinetic studies [J]. Nat Prod Res, 2012, 26: 530-539.

[18] Han XZ, Gao S, Cheng YN, et al. Protective effect of naringenin-7-*O*-glucoside against oxidative stress induced by doxorubicin in H9c2 cardiomyocytes [J]. Biosci Trends, 2012, 6: 19-25.

[19] Raafat K, Wurglics M, Schubert-Zsilavecz M. *Prunella vulgaris* L. active components and their hypoglycemic and antinociceptive effects in alloxan-induced diabetic mice [J]. Biomed Pharmacother, 2016, 84: 1008-1018.

[20] Chen L, Tang ZS, Song ZX, et al. Quantitative determination of nine furanocoumarins for quality evaluation of *Angelica dahurica* from different habitats [J]. China J Chin Mater Med (中国中药杂志), 2019, 44: 3002-3009.

[21] Liang CJ, Yin JT, Ma YL, et al. Quantitative determination of characteristic components from compound of *Lysionotus pauciflorus* Maxim. by LC-MS/MS and its application to a pharmacokinetic study [J]. J Pharm Biomed Anal, 2020, 177: 112835-112844.

[22] Wang YJ, Wen J, Zheng WH, et al. Simultaneous determination of neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid and geniposide in rat plasma by UPLC-MS/MS and its application to a pharmacokinetic study after administration of Reduning injection [J]. Biomed Chromatogr, 2015, 29: 68-74.

[23] Zhan Y, Li R, Li XL, et al. Chlorogenic acid down-regulates the expression of PD-L1 in esophageal squamous cell carcinoma *via* IFN-*γ* signaling pathway [J]. Acta Pharm Sin (药学学报), 2021, 56: 1599-1605.

[24] Montero L, Ibáñez E, Russo M, et al. Metabolite profiling of licorice (*Glycyrrhiza glabra*) from different locations using comprehensive two-dimensional liquid chromatography coupled to diode array and tandem mass spectrometry detection [J]. Anal Chim Acta, 2016, 913: 145-159.

[25] Li J, Chen JL, Ma YG, et al. Determination of components of Sparganii Rhizoma absorbed into blood by HPLC [J]. Chin J Mod Appl Pharm (中国现代应用药学), 2019, 36: 1665-1668.

[26] Du YY, He BS, Li Q, et al. Identification and analysis of chemical constituents and rat serum metabolites in Suan-Zao-Ren gran-

ule using ultra high performance liquid chromatography quadrupole time-of-flight mass spectrometry combined with multiple data processing approaches [J]. J Sep Sci, 2017, 40: 2914-2924.

[27] Peng C, Zhou ZM, Li J, et al. CCl_4-induced liver injury was ameliorated by Qi-Ge Decoction through the antioxidant pathway [J]. Evid Based Complement Alternat Med, 2019, 2019: 5941263-5941275.

[28] Ruan YD, Huang ZH, Chen ML, et al. Research on mechanism of Biyuan Tongqiao granules in the treatment of rhinitis based on network pharmacology and molecular docking [J]. Eva Anal Drug Use Hosp China (中国医院用药评价与分析), 2021, 21: 897-904, 907.

[29] Niu YX, She ZF, Su CH, et al. The effects and the mechanisms of naringenin from *Artemisia ordosica* Krasch on allergic rhinitis based on mast cell degranulation model and network pharmacology [J]. J Pharm Pharmacol, 2022, 74: 397-408.

[30] Yang SS, Fu QW, Deng H, et al. Mechanisms and molecular targets of the Yu-Ping-Feng powder for allergic rhinitis, based on network pharmacology [J]. Medicine (Baltimore), 2021, 100: e26929.

[31] Qi YY, Han XL, Wang QQ, et al. Study on the mechanism of Buzhong Yiqi decoction in the treatment of allergic rhinitis based on network pharmacology [J]. World Chin Med (世界中医药), 2021, 16: 2987-2991, 2998.

[32] Sakai H, Hara T, Todoroki K, et al. Elevated guanylate cyclase and cyclic-guanosine monophosphate-dependent protein kinase levels in nasal mucosae of antigen-challenged rats [J]. Microvasc Res, 2013, 90: 150-153.

[33] Zhang MJ, Zhou J, Wang L, et al. Caffeic acid reduces cutaneous tumor necrosis factor alpha (TNF-α), IL-6 and IL-1β levels and ameliorates skin edema in acute and chronic model of cutaneous inflammation in mice [J]. Biol Pharm Bull, 2014, 37: 347-354.

[34] Wu YJ, Su J, Huang PJ, et al. Buddleoside prevents TNF-α-induced human aortic endothelial cells inflammatory injury through inhibiting TLR4/IκBα/NF-κB signaling pathway [J]. Chin J Mod Appl Pharm (中国现代应用药学), 2017, 34: 637-643.

[35] Jiang M, Zhou MG, Han YQ, et al. Identification of NF-κB Inhibitors in Xuebijing injection for sepsis treatment based on bioactivity-integrated UPLC-Q/TOF [J]. J Ethnopharmacol, 2013, 147: 426-433.

[36] Jiang YQ, Tang QF, Zhang S, et al. Combined effects of gallic acid and ciprofloxacin on the murine chronic rhino-sinusitis model in mice [J]. J China Pharm Univ (中国药科大学学报), 2015, 46: 600-604.

[37] Chen N, Wu QC, Chi GF, et al. Prime-*O*-glucosylcimifugin attenuates lipopolysaccharide-induced acute lung injury in mice [J]. Int Immunopharmacol, 2013, 16: 139-147.

[38] Xu K, Gao Y, Yang L, et al. Magnolin exhibits anti-inflammatory effects on chondrocytes *via* the NF-κB pathway for attenuating anterior cruciate ligament transection-induced osteoarthritis [J]. Connect Tissue Res, 2021, 62: 475-484.

[39] Pei K, Ning Y, Cai H, et al. Mechanism analysis of strengthening anti-cerebral ischemia injury of Chuanxiong Rhizoma processed with wine based on UHPLC-Q-orbitrap HRMS and integrated network pharmacology [J]. Chin J Exp Tradit Med Form (中国实验方剂学杂志), 2022, 28: 164-173.

[40] She XH, Feng LP, Huang YM, et al. Potential molecular mechanism of Simiao Sini Decoction in treatment of chronic cervicitis based on network pharmacology and molecular docking technology [J]. Clin J Tradit Chin Med (中医药临床杂志), 2022, 34: 682-686.

[41] Zhou LL, Han ZJ, Zhang RX, et al. Protective effect of rosmarinic acid on oxidative stress reaction of allergic rhinitis induced by PM2.5 [J]. Chin J Ophthalmol Otorhinolaryngol (中国眼耳鼻喉科杂志), 2021, 21: 276-281.

[42] Cao W, Wei SJ, Zhang WJ, et al. Anti-tumor effect and the mechanism of inducing apoptosis of rosmarinic acid on liver cancer-bearing mice [J]. J Guangxi Med Univ (广西医科大学学报), 2020, 37: 405-410.