红花逍遥片的化学成分谱鉴定及其干预经前期综合征“病−证−症−方”关联的功效内涵解析

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摘要: 鉴定红花逍遥片的化学成分谱, 从“病−证−症−方”关联角度, 探索该中成药品种治疗经前期综合征 (premenstrual syndrome, PMS) 肝郁气滞血瘀证的作用特点与生物学内涵。采用超高效液相色谱−四极杆/静电场轨道阱高分辨质谱 (UHPLC-Q Exactive Orbitrap HRMS) 鉴定红花逍遥片的化学成分谱; 依据组方原理将其划分为疏肝解郁组、活血调经组和益气健脾组, 通过Pharmmapper数据库与中医药整合药理学研究平台 (TCMIP v2.0) 收集红花逍遥片各功效组所含中药的候选靶标信息; 通过中医证候本体及多维定量关联计算平台 (SoFDA) 数据库、GeneCards、DisGeNET、MalaCards和已发表文献, 收集PMS现代医学的临床症状、中医诊疗标准及其临床症状的相关基因集; 依据基因间相互作用信息, 建立“红花逍遥片候选靶标−PMS肝郁气滞血瘀证相关基因”互作网络, 通过计算网络拓扑特征值, 筛选核心网络靶标, 并基于Kyoto Encyclopedia of Genes and Genomes (KEGG) 数据库开展功能挖掘, 进一步探索红花逍遥片组方中各功效组在干预PMS中的优势药效环节, 并加以动物实验验证。红花逍遥片中共鉴定获得109个化学成分, 其中疏肝解郁组含20个化学成分, 主要作用于神经系统、雌激素调节与“免疫−炎症”相关通路而发挥疏肝功效; 活血调经组含77个化学成分, 主要作用于血液循环系统、“免疫−炎症”与雌激素调节相关通路而发挥补血和气、养血柔肝的功效; 益气健脾组含30个化学成分, 通过调节“免疫−炎症”、消化系统与激素水平而发挥扶正益气、利水渗湿的功效。疾病靶组织生化指标检测结果表明, 与溶剂对照组相比, PMS模型大鼠下丘脑组织中的5-HT与DA水平下降, 子宫组织中E_2、NO、VEGF、RLN水平下降, OT、PROG、IL-6、IL-1β、TNF-α、ET-1水平上升, 红花逍遥片给药后能够缓解或逆转PMS大鼠的上述病理改变, 表明红花逍遥片能够通过调节雌激素合成与分泌、干预神经递质合成与信号传导、矫正“免疫−炎症”失衡和调节消化系统功能, 发挥其舒肝、理气、活血、健脾的综合功效, 有助于改善PMS肝郁气滞血瘀证中机体“神经−内分泌−免疫”系统与血液循环紊乱, 为明确红花逍遥片治疗PMS肝郁气滞血瘀证的优势药效环节和探索其作用机制提供参考。本实验获得中国中医科学院中药研究所动物伦理委员会批准 (批准号: 2023B248)。

关键词: 红花逍遥片; 经前期综合征; “病−证−症−方”关联网络; UHPLC-Q Exactive Orbitrap HRMS; 肝郁气滞; 血瘀

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Identification of chemical constituents in Honghua Xiaoyao Tablet and the analysis of efficacy connotation against premenstrual syndrome based on the "disease-syndrome-symptom-formula" association network

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Abstract: The present study identified chemical constituents of Honghua Xiaoyao Tablet (HXT) and explored

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its biological connotation and characteristics on the premenstrual syndrome (PMS) treatment from the "disease-syndrome-symptom" association network. UHPLC-Q Exactive Orbitrap HRMS technology was applied to analyze the chemical constituents in HXT. According to the composition principles, the compatible herbs of HXT were divided into the Shugan Jieyu group, Huoxue Tiaojing group and Yiqi Jianpi group. The candidate targets of the corresponding prescriptions of HXT efficacy groups were collected from the Pharmmapper database and Integrative Pharmacology-based Research Platform of Traditional Chinese Medicine (TCMIP) v2.0. The gene set related to the clinical symptoms included in Traditional Chinese and Western Medicine diagnosis and treatment standards were obtained from SoFDA, GeneCards, DisGeNET, MalaCards and literature published. The "HXT candidate targets-PMS (liver depression, Qi stagnation, and blood stasis syndrome) genes" network was constructed based on the gene interaction information, and further, the core network targets were screened out by topological characteristics of calculating network, and the functional exploration was carried out based on Kyoto Encyclopedia of Genes and Genomes (KEGG) for exploring the therapeutic advantages in PMS treatment of HXT efficacy groups, which were further verified experimentally *in vitro*. A total of 109 components from HXT were identified, including 20 components from Shugan Jieyu group enriched in the neurological system, estrogen regulation, and "immune-inflammation" related pathways, 77 components from Huoxue Tiaojing group enriched in the blood-circulation system, "immune-inflammation" and estrogen regulation related pathways and 30 components from Yiqi Jianpi group regulating immune inflammation, digestive system, and hormone levels. The biochemical indicator detection demonstrated that both the levels of 5-HT and DA in the hypothalamus tissues and the levels of E_2, NO, VEGF and RLN in the uterine tissues of PMS model rats were lower than those in controls, which the levels of OT, PROG, IL-6, IL-1β and TNF-α in the uterine tissues were increased in PMS group, which were all reversed by the administration of HXT, indicating that this prescription may regulate the synthesis and secretion of estrogen, intervene in neurotransmitter synthesis and signal transduction, reverse the imbalance of "immune-inflammation", and regulate digestive system function through various biological pathways, exerting the liver-smoothing, Qi-regulating, blood-activating and spleen-tonifying comprehensive effects, leading to alleviating the "neuroendocrine-endocrine-immune" system and blood-circulation disorders. The relevant results may provide a reference for clarifying the advantages and efficacy of HXT in treating PMS with liver stagnation, Qi stagnation, and blood stasis syndrome, and exploring its therapeutic advantages. The animal experiment of this study was approved by the Ethics Committee of the Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences (approval number: 2023B248).

Key words: Honghua Xiaoyao Tablet; premenstrual syndrome; "disease-syndrome-symptom-formula" association network; UHPLC-Q Exactive Orbitrap HRMS; liver depression and Qi stagnation syndrome; blood stasis syndrome

逍遥类方剂被誉为“妇科圣药”, 是中医治疗抑郁症的常用方剂, 其中, 红花逍遥片是以其为基础, 结合云南哀牢山区彝族传统医学文献《哀牢本草》[1], 科学组方而成的中成药, 由当归、白芍、白术、茯苓、红花、皂角刺、竹叶柴胡、薄荷、甘草组成, 具有舒肝、理气、活血的功效, 已广泛用于更年期综合征、乳腺炎、原发性痛经、围绝经期综合征、黄褐斑[1-3]等妇科疾病的治疗。经前期综合征 (premenstrual syndrome, PMS) 是一种严重威胁女性身心健康的神经内分泌系统疾病, 属于中医“月经前后诸证”范畴, 病因病机复杂, 由肝失疏泄为主要病因, 累及诸多脏腑、经络, PMS发病机制与临床症状的繁杂, 导致目前尚未形成行之有效的诊治方案, 如PMS一线治疗药物5-羟色胺再摄取抑制剂 (SSRIs) 不能完全缓解近75%的患者症状, 并伴随诸多不良反应[4]。已有临床研究[1]表明, 红花逍遥片在治疗以肝郁气滞血瘀为主要证型的PMS中具有良好疗效, 但其治疗PMS的物质基础、疗效优势和功效内涵均未见深入研究与报道。

本研究依据组方原则对红花逍遥片进行功效组划分, 并通过超高效液相色谱−四极杆/静电场轨道阱高分辨质谱 (UHPLC-Q Exactive Orbitrap HRMS) 定性分析红花逍遥片体外成分谱, 建立和挖掘“功效组−化学成分−药物靶标−疾病/证候/症状基因”多层次关联网络, 初步揭示红花逍遥片各功效组药理作用特点及其对PMS肝郁气滞血瘀证的治疗潜能与优势疗效环节, 并加以动物实验验证, 以期为探索与阐释红花逍遥片干预PMS肝郁气滞血瘀证的潜在药效成分、优势疗效环节及其中医科学内涵奠定基础, 并为红花逍遥片的

临床精准对证与合理用药提供支撑。

材料与方法

仪器 Thermo Q-Extractive液相色谱–四极杆–静电场轨道阱高分辨质谱联用仪、Xcalibur数据分析系统 (美国Thermo公司); Waters ACQUITY UPLC BEH C18色谱柱 (130 Å, 1.7 μm, 2.1 mm×150 mm, 美国Waters公司); 十万分之一电子天平 (瑞士梅特勒–托利多有限公司, MS105 Semi-Micro型); MK3全自动酶标仪 (美国Thermo Fisher Scientific公司)。

药物与试剂 红花逍遥片原料药 (江西普正药业, 规格: 每1 g原料药相当于成药3.4片); 坤泰胶囊 (贵州新天药业, 批号: 230711); 乙腈 (质谱级)、甲醇 (色谱纯), 美国Thermo Fisher Scientific公司; 甲酸 (HPLC级, 美国Sigma Aldrich公司); 蒸馏水 (广州屈臣氏食品饮料有限公司)。黄体酮 (货号: s30586, 上海源叶生物科技有限公司); 芝麻油 (货号: is9030, 北京索莱宝科技有限公司); 生理盐水 (货号: G4702, 武汉赛维尔生物科技有限公司); 5-羟色胺 (5-hydroxytryptamine, 5-HT, 货号: ml059511)、多巴胺 (dopamine, DA, 货号: ml003201)、雌二醇 (estradiol, E_2, 货号: ml002871)、黄体酮 (progesterone, PROG, 货号: ml002894)、白介素6 (interleukin-6, IL-6, 货号: ml102828)、白介素1β (interleukin-1β, IL-1β, 货号: ml003057)、肿瘤坏死因子-α (tumor necrosis factor-α, TNF-α, 货号: ml002859)、内皮素-1 (endothelin-1, ET-1, 货号: ml002890)、血管内皮生长因子 (vascular endothelial growth factor, VEGF, 货号: ml002862)、催产素 (oxytocin, OT, 货号: ml037162)、松弛素 (relaxin, RLN, 货号: ml303972) 实验大鼠ELISA试剂盒和NO试剂盒 (货号: ml076493) 购自上海酶联生物科技有限公司。

数据库及软件 红花逍遥片候选靶标收集: PubChem数据库 (https://pubchem.ncbi.nlm.nih.gov/)、ETCM 2.0 (http://www.tcmip.cn/ETCM2/)、PharmMapper数据库 (http://www. lilab-ecust. cn/pharmmapper/)、Uniprot数据库 (https://www.uniprot.org/)。

PMS相关基因集的收集: PharmMapper数据库 (http://www. lilab-ecust. cn/pharmmapper/)、GeneCards (https://www.genecards.org/)、DisGeNET (www.DisGeNET.org) 和MalaCards (https://www.malacards.org/)、中医证候本体及多维定量关联计算平台[5] (SoFDA, http://www.tcmip.cn/Syndrome/)。

其他软件及数据库: STRING v11.0平台 (https://www. string-db. org/)、DAVID v6.8 (http://david. abcc.ncifcrf.gov/)、Cytoscape v3.10.0、R v3.5.3。

红花逍遥片组方理论与功效组划分 红花逍遥片由白芍、当归、茯苓、白术、皂角刺、红花、柴胡、薄荷、甘草九味药组成, 方中竹叶柴胡为君药, 有疏肝解郁、疏风退热之效。当归、白芍、茯苓、白术为臣药, 其中当归补血活血、调经止痛, 白芍养血调经、柔肝敛阴, 茯苓利水渗湿、健脾生津、宁心安神, 白术健脾益气、燥湿利水。薄荷、皂角刺与红花为佐药, 薄荷能够疏散风热、疏肝行气, 化解肝郁而生之热, 皂角刺消肿托毒, 红花活血通经、散瘀止痛。甘草为使药, 补中益气、缓急止痛, 同时调和诸药。整体组方配伍能够有效理气疏肝、解郁健脾、祛瘀活血、散结化痰、舒经通络、补益气血。诸药联合使用共奏疏肝、理气、解郁、活血之功效。依据上述组方理论与功效划分, 竹叶柴胡与薄荷能够疏肝、行气、散热, 故将其归为疏肝解郁组[6], 红花、当归、白芍、皂角刺能够养血调经、活血散瘀, 故将其归为活血调经组, 白术、茯苓、甘草能够益气健脾、利水渗湿, 故将其归为益气健脾组, 这一功效划分标准既能反映红花逍遥片组方特色, 又能凸显其临床对证原则。

红花逍遥片待测样品制备 取红花逍遥片粉末适量, 加80%甲醇溶液超声提取30 min, 制成质量浓度为10 mg·mL^{-1}溶液, 取0.22 μm超滤膜过滤续滤液待测。

UHPLC-Q Exactive Orbitrap HRMS检测条件 液相色谱条件: 流动相A为0.1%甲酸水, 流动相B为乙腈。色谱梯度洗脱条件设置如下: 95% A, 0～3 min; 95%～84% A, 3～10 min; 85%～78% A, 10～30 min; 78%～75% A, 30～35 min; 75%～67% A, 35～45 min; 67%～60% A, 45～55 min; 60%～50% A, 55～65 min; 50%～35% A, 65～76 min。流速0.3 mL·min^{-1}, 柱温25 ℃。进样量设置为4 μL, 流速设置为0.30 mL·min^{-1}。紫外检测波长235 nm。质谱检测器条件: 采用电喷雾电离 (ESI) 离子源, 正、负离子模式采集数据。数据采集范围*m/z*设置为100～1 500。裂解电压 (DP): 100 V, 碰撞能量 (CE): 25 eV, 碰撞电压差 (CES): 15 eV。

UHPLC-Q Exactive Orbitrap HRMS数据分析 利用Xcalibur软件, 对比文献资料分析不同类型化合物的质谱裂解规律, 分析红花逍遥片中各化合物的保留时间、分子式、精确相对质量和碎片离子。

红花逍遥片候选靶标谱获取 通过中医药整合药理学研究平台 (TCMIP v2.0) 与Pharmmapper数据库 (http://www.lilab-ecust.cn/pharmmapper/) 收集红花逍遥片的候选靶标, 设定相似性评分 (similar score) ≥ 0.8或拟合度 (normal fit) ≥ 0.9, 利用UniProt蛋白质数据库, 将候选靶标蛋白质名称转换为官方基因名称 (official gene symbol), 种属设置为“Human”。

PMS相关基因集的收集和整理 以“premenstrual dysphoric disorder”、“premenstrual syndrome”和“premenstrual tension”为关键词, 在GeneCards (https://www.genecards.org/)、DisGeNET (www.DisGeNET.org)和MalaCards (https://www.malacards.org/) 等数据库收集PMS相关基因。其中, GeneCards数据库基因以relevance score ⩾ 10为标准进行筛选, MalaCards数据库基因参考数据库引用文献内容进行筛选。收集文献[7]中基于转录组学的疾病相关的差异基因表达信息, 通过R语言biomaRt函数进行小鼠与人类之间的同源基因转换。基于MalaCards数据库收集临床批准的治疗PMS药物, 分为选择性SSRIs、前列腺素抑制剂、雄激素抑制剂、雌激素、抗焦虑剂、避孕药、肾上腺皮质激素类药、阿片受体激动剂、促性腺激素释放激素增效剂 (GnRH-a)、维生素、醛固酮受体拮抗剂类, 并通过PharmMapper数据库预测药物候选靶标, 同类药物靶点取交集, 各类药物基因集整合去冗余后, 得到PMS相关基因集。

证候相关基因集的收集 以“肝郁气滞”与“血瘀”为关键词, 通过SoFDA平台筛选证候相关的中医证候靶标。

症状相关基因集的收集和整理 参考《中医妇科常见病诊疗指南》(2012年) PMS肝郁气滞证辨证标准: 主症: ① 经前乳房和 (或) 乳头胀痛, ② 小腹胀满或连及胸胁, ③ 烦躁易怒或精神抑郁; 次症: ① 头晕, ② 失眠, ③ 头痛剧烈, ④ 经来不畅、色暗红。通过GeneCards数据库收集关键词为“depression”、“irritability”、“vertigo”、“headache”、“abdominal pain”、“breast lump”、“breast tenderness”、“swelling”和“insomnia”的基因, 选择其中relevance score ⩾ 10的部分作为PMS的症状表型基因。

网络拓扑特征值计算和关键网络靶标功能挖掘 提取PMS疾病、症状及证候基因的并集, 通过STRING v11.0平台 (https://www.string-db.org/) 提取上述节点基因相互作用信息, 进而构建“疾病−证候−症状”相互作用网络, 通过Cytoscape 3.10.0软件对网络进行分析, 计算度值 (degree)、介数中心性 (betweenness centrality)、接近中心性 (closeness centrality) 的网络拓扑特征值, 以中位数为卡值, 选择同时大于这3个指标中位数的靶标, 筛选出PMS核心靶标。通过DAVID v6.8在线生物信息分析平台 (http://david.abcc.ncifcrf.gov/) 对其生物功能进行注释和富集分析 (显著性标准: $P < 0.05$ 且Benjamini校正 $P < 0.05$), 以挖掘PMS病证结合的生物内涵。

红花逍遥片功效组网络调控机制研究 整理PMS疾病、症状及证候基因以及红花逍遥片候选靶标, 删去冗余部分, 通过STRING v11.0平台 (https://www.string-db.org/) 提取上述节点基因相互作用信息, 进而构建“功效组药物靶标−疾病基因−证候基因−症状基因”互作网络, 通过Cytoscape 3.10.0软件对网络进行分析, 计算度值、介数中心性、接近中心性的网络拓扑特征值, 以中位数为卡值, 选择同时大于这3个指标中位数的靶标, 筛选出红花逍遥片治疗PMS的核心靶标。通过DAVID v6.8在线生物信息分析平台 (david.abcc.ncifcrf.gov/) 对其生物功能进行注释和富集分析 (显著性标准: $P < 0.05$ 且Benjamini校正 $P < 0.05$), 以挖掘红花逍遥片治疗PMS的生物学功能。

实验动物分组及处理 SPF级雌性Wistar大鼠73只, 6～8周龄, 160～180 g, 购于维通利华 (北京) 生物技术有限公司 [许可证编号: SYXK (京) 2019-0003]。动物饲养于中国中医科学院中药研究所实验动物中心SPF标准环境下。日光灯循环进光照, 温度、湿度适宜动物生长。实验动物生产许可证号: SCXK (京) 2021-0006, 实验动物质量合格证编号: No.110011231111067061。本实验获得中国中医科学院中药研究所动物伦理委员会批准 (批准号: 2023B248)。

将实验动物随机分为空白对照组 (8只)、溶剂对照组 (8只)、模型组 (11只)、阳性药组 (11只, 540 mg·kg^{-1})、低剂量组 (11只, 210 mg·kg^{-1})、中剂量组 (11只, 420 mg·kg^{-1}) 和高剂量组 (11只, 840 mg·kg^{-1}) 共7组。适应性喂养1周后, 模型组、阳性药组、红花逍遥片低剂量组、中剂量组和高剂量组每只大鼠腹腔注射0.2 mL黄体酮芝麻油混悬液 (30 mg·mL^{-1}), 空白对照组与溶剂对照组分别注射等体积生理盐水与芝麻油。连续注射7天后进行灌胃给药, 第21天停止造模与给药, 48 h后取各组大鼠子宫与下丘脑组织待测。

生化指标检测 根据试剂盒说明书, 通过酶联免疫吸附测定法检测各组大鼠下丘脑组织5-HT、DA水平, 子宫组织E_2、PROG、IL-6、IL-1β、TNF-α、ET-1、NO、VEGF、OT、RLN水平。

结果

1 红花逍遥片体外成分谱鉴定

红花逍遥片供试品正、负离子模式下采集, 如表1[8-33]所示, 初步鉴定出109个化合物, 通过搜索其相对保留时间、分子式、母离子和离子碎片, 并将质量误差控制在10 ppm以内, 对各鉴定化合物的母离子峰、离子碎片、分类及化合物归属等信息进行总结。其中, 鉴定获得当归所含化学成分15个、白芍所含化学成分

36个、白术所含化学成分7个、茯苓所含化学成分8个、红花所含化学成分17个、皂角刺所含化学成分9个、竹叶柴胡所含化学成分12个、薄荷所含化学成分8个和甘草所含化学成分15个。

Table 1 Compounds detected in full MS mode were identified in Honghua Xiaoyao Tablet (HXT) extraction based on MS^n data. DG: Angelicae Sinensis Radix; BS: Paeoniae Radix Alba; BZ: Atractylodes macrocephala Rhizoma; FL: Poria cocos; HH: Carthami Flos; ZJC: Gleditslae Spina; ZYCH: Bupleurum bamboos; BH: Menthae Haplocalycis Herba; GC: Glycyrrhizae Radix et Rhizoma

RT/min	Component	Formula	Formula	Calculated mass	Measured mass	MS/MS	Source	Reference
0.74	Arginine	$C_6H_{14}N_4O_2$	$[M+H]^+$	175.119 0	175.118 6	116.070 7, 131.082 6	BZ, DG, BS	[8-10]
0.81	Benzoyl paeoniflorin	$C_{16}H_{24}O_{10}$	$[M-H]^-$	375.129 7	375.126 9	195.063 6	BS	[10]
0.88	Sucrose	$C_{12}H_{22}O_{11}$	$[M-H]^-$	341.108 9	341.109 4	179.055 2, 149.046 0, 131.034 9	BS	[10]
1.20	Tyrosine	$C_9H_{11}NO_3$	$[M-H]^-$	182.081 1	182.080 2	136.075 5	BS	[10]
1.21	Adenosine	$C_{10}H_{13}N_5O_4$	$[M+H]^+$	268.104 0	268.117 8	136.061 6	BS, DG	[8,9]
1.35	1-*O*-Galloyl-beta-*D*-glucose	$C_{13}H_{16}O_{10}$	$[M-H]^-$	331.067 1	331.067 7	169.014 4	BS	[10]
1.47	Gallic acid	$C_7H_6O_5$	$[M-H]^-$	169.014 2	169.014 4	125.024 5, 124.016 8	BS	[11]
1.81	Isoferulic acid	$C_{10}H_{10}O_4$	$[M]^+$	194.057 9	194.080 7	151.095 9	DG	[9]
1.85	Pyrogallol	$C_6H_6O_3$	$[M-H]^-$	125.024 4	125.024 5	107.013 8, 97.029 5, 81.034 5	BS	[12]
1.95	Ethyl gallate	$C_9H_{10}O_5$	$[M-H]^-$	197.045 5	197.045 8	153.019 2, 125.022 2, 124.040 5	BS	[8]
2.43	*p*-Hydroxybenzaldehyde	$C_7H_6O_2$	$[M+H]^+$	123.044 4	123.055 2	100.024 4, 81.070 3	HH	[13]
2.54	Senkyunolide H	$C_{12}H_{16}O_4$	$[M+H]^+$	225.112 5	225.112 1	207.062 5	DG	[9]
2.85	Fructose/Glucose	$C_6H_{12}O_6$	$[M-H]^-$	179.056 1	179.058 1	179.055 7	BS	[10]
2.90	Catechin	$C_6H_6O_3$	$[M+H]^+$	127.039 0	127.038 9	109.028 6, 81.034 1	BZ	[14]
3.75	7-Hydroxycoumarin	$C_{15}H_{14}O_6$	$[M-H]^-$	289.071 8	289.090 9	244.888 0, 179.117 4	BS	[11]
3.92	Umbelliferone	$C_9H_6O_3$	$[M+H]^+$	163.039 0	163.038 6	145.028 2, 135.043 9, 117.033 5, 107.049 3, 89.039 0	BZ	[15]
4.75	Protocatechualdehyde	$C_7H_6O_3$	$[M-H]^-$	137.024 4	137.024 5	109.029 5	BS	[10]
5.63	3-Methoxygallic acid	$C_8H_8O_5$	$[M-H]^-$	183.029 9	183.029 9	168.006 4, 154.998 6, 139.003 6, 124.016 5, 111.008 8	BS	[8]
5.71	Protocatechuic acid	$C_7H_6O_4$	$[M-H]^-$	153.019 3	153.019 4	109.029 6	BS, DG	[16]
6.59	Vanillin	$C_8H_8O_3$	$[M-H]^-$	151.040 1	151.040 2	125.035 6, 111.009 1, 65.014 5	ZJC	[17]
7.48	Vanillic acid	$C_8H_8O_4$	$[M-H]^-$	167.035 0	167.035 0	152.011 6	DG	[16]
7.51	Benzoic acid	$C_7H_6O_2$	$[M-H]^-$	121.029 5	121.029 6	93.034 6	BS	[8]
7.54	Caffeic acid	$C_9H_8O_4$	$[M-H]^-$	179.035 0	179.033 6	135.080 0	BH	[18]
7.60	Paeonilactone B	$C_{10}H_{12}O_4$	$[M+H]^+$	197.080 8	197.080 6	179.06 9, 151.038 8, 123.043 9	BS	[8]
8.08	Phenylalanine	$C_9H_{11}NO_2$	$[M-H]^-$	164.070 2	164.071 7	167.046 8	DG	[9]
8.66	Senkyunolide F	$C_{12}H_{14}O_3$	$[M+H]^+$	207.101 7	207.101 6	189.127 0, 161.132 1	DG	[9]
8.69	Roseoside	$C_{19}H_{30}O_8$	$[M+Na]^+$	409.183 3	409.182 5	391.188 5, 203.052 9	HH	[19,20]
8.71	Isopaeoniflorin	$C_{23}H_{27}O_{11}$	$[M+HCOO]^-$	525.160 2	525.161 8	479.216 8, 121.029 6	BS	[8]
8.91	Paeoniflorin sulfite	$C_{23}H_{28}O_{13}S$	$[M-H]^-$	543.117 8	543.118 3	421.081 3, 375.076 9, 259.028 4, 213.022 6, 121.029 6	BS	[12]
9.25	Hydroxysafflor yellow A	$C_{27}H_{32}O_{16}$	$[M+H]^+$	613.176 3	613.263 4	451.121 6, 433.112 2, 331.080 9, 301.069 5	HH, DG	[16]
9.35	Cytidine	$C_9H_{13}N_3O_5$	$[M+H]^+$	244.092 8	244.096 3	226.105 9	HH	[21]
9.67	Paeonol	$C_9H_{10}O_3$	$[M-H]^-$	165.055 7	165.055 8	139.051 1, 121.029 5, 107.025 2	BS	[8]
10.12	Oxypaeoniflorin	$C_{23}H_{28}O_{12}$	$[M-H]^-$	495.150 8	495.151 6	345.119 8, 333.097 7, 165.055 6, 137.024 4, 97.029 5	BS	[11]
10.32	Paeonilactone C	$C_{17}H_{18}O_6$	$[M+H]^+$	319.117 6	319.117 3	301.107 6, 273.075 0, 197.080 5, 151.075 0	BS	[8]
10.41	*p*-Coumaric acid	$C_9H_8O_3$	$[M-H]^-$	163.040 1	163.040 2	119.050 3	ZJC	[22]
10.96	Albiflorin	$C_{23}H_{28}O_{11}$	$[M+HCOO]^-$	525.161 6	525.161 4	479.156 1, 449.144 5, 357.119 5, 327.108 2, 121.029 6	BS	[12]
11.03	Galloylpaeoniflorin	$C_{30}H_{32}O_{15}$	$[M-H]^-$	631.166 8	631.167 7	313.056 9, 169.014 3, 125.024 4	BS	[10]
11.12	Paeoniflorin	$C_{23}H_{28}O_{11}$	$[M+HCOO]^-$	524.154 1	524.149 2	165.020 3, 121.029 5	BS	[8,23]
11.68	Ferulic acid	$C_{10}H_{10}O_4$	$[M-H]^-$	193.050 6	193.049 3	149.059 6, 137.059 5	DG	[9]
11.88	6-Hydroxy kaempferol	$C_{15}H_{10}O_7$	$[M+H]^+$	303.049 9	303.040 4	285.123 5, 257.033 1, 165.052 6	HH	[24]
11.98	Adenine	$C_5H_5N_5$	$[M+H]^+$	136.061 8	136.075 5	135.117 1, 118.065 2, 108.081 1	HH, ZJC	[17,25]
12.03	Kaempferol	$C_{15}H_{10}O_6$	$[M+H]^+$	287.055 0	287.056 6	241.050 6, 213.054 2, 153.019 7	HH, ZJC	[17,24]
12.81	Kaempferol 3,7-di-*O*-*β*-*D*-glucoside	$C_{27}H_{30}O_{16}$	$[M+H]^+$	611.160 7	611.160 3	593.214 8, 449.110 4, 287.054 4	BS	[8]
12.91	Tilianin	$C_{22}H_{22}O_{10}$	$[M+H]^+$	447.128 5	447.122 6	225.054 0	BH	[18]
13.00	Ellagic acid	$C_{14}H_6O_8$	$[M-H]^-$	300.999 0	300.999 4	257.010 8, 229.014 3, 185.024 5	BS	[11]

Continued

RT/min	Component	Formula	Formula	Calculated mass	Measured mass	MS/MS	Source	Reference
13.03	Rutin	$C_{27}H_{30}O_{16}$	$[M-H]^-$	609.146 1	609.147 1	300.027 8, 151.003 9	HH, ZJC, ZYCH	[17,24]
13.08	Hyperoside	$C_{21}H_{20}O_{12}$	$[M+H]^+$	465.102 8	465.100 4	303.049 2	HH, ZYCH	[24]
13.18	Atractylenolide III	$C_{15}H_{20}O_3$	$[M+H]^+$	249.148 5	249.148 9	231.100 5	BZ	[14]
13.47	Quercetin	$C_{15}H_{10}O_7$	$[M+H]^+$	303.049 9	303.051 6	285.040 7, 257.045 3, 165.055 6, 153.019 4	ZYCH, HH, BS, ZJC	[8,24]
13.53	Salicylic acid	$C_7H_6O_3$	$[M-H]^-$	137.024 4	137.024 6	119.025 0, 93.346 0	BS, ZJC	[8,17]
13.81	Isoquercitrin	$C_{21}H_{20}O_{12}$	$[M-H]^-$	463.088 2	463.089 1	271.025 1, 178.998 7, 151.003 7	HH, ZJC, ZYCH	[17,26]
14.12	Liquiritin apioside	$C_{26}H_{30}O_{13}$	$[M-H]^-$	549.161 4	549.161 4	297.038 0, 255.064 6	GC	[27]
14.16	Liquiritigenin	$C_{15}H_{12}O_4$	$[M+H]^+$	257.080 8	257.080 2	257.080 2, 163.038 8, 137.023 1, 119.049 1, 91.054 5	GC	[27]
14.18	Isoliquiritin	$C_{21}H_{22}O_9$	$[M-H]^-$	417.119 1	417.115 8	255.064 7, 148.016 7, 135.134 0, 119.049 1, 91.054 7	GC	[27]
14.91	Eriodictiol	$C_{15}H_{12}O_6$	$[M+H]^+$	289.070 6	289.069 9	271.094 6, 168.101 8	HH	[13]
14.98	Didymin	$C_{28}H_{34}O_{14}$	$[M+H]^+$	595.202 1	595.215 7	287.054 4	BH	[18]
15.72	Quercitrin	$C_{21}H_{20}O_{11}$	$[M-H]^-$	447.093 3	447.093 7	300.060 0, 255.030 3, 151.003 3	HH, ZJC	[17]
15.93	6'-*O*-Galloylalbiflorin	$C_{30}H_{32}O_{15}$	$[M-H]^-$	631.166 8	631.168 2	313.057 0, 169.014 3, 125.024 5	BS	[10]
16.05	Apigenin	$C_{15}H_{10}O_5$	$[M+H]^+$	271.060 1	271.059 6	153.069 7, 243.064 0	HH	[24]
16.25	Naringenin	$C_{15}H_{12}O_5$	$[M-H]^-$	271.060 1	271.060 1	177.065 5, 151.039 0, 119.028 4	BS, GC	[8]
16.46	Scopoletin	$C_{10}H_8O_4$	$[M+H]^+$	193.049 5	193.049 2	161.059 8, 133.064 8	BZ	[8]
18.00	Diosmin	$C_{28}H_{32}O_{15}$	$[M+H]^+$	609.181 4	609.178 0	301.070 1	BH	[18]
18.46	Rosmarinic acid	$C_{18}H_{16}O_8$	$[M-H]^-$	359.077 2	359.077 6	161.024 5, 135.046 2, 123.045 2, 133.029 4	HH	[25]
19.32	5-Hydroxyliquiritin	$C_{21}H_{22}O_{10}$	$[M-H]^-$	433.114 0	433.114 1	271.059 4, 177.018 3, 151.038 5, 119.049 1	GC	[27]
21.14	Chrysophanol	$C_{15}H_{10}O_4$	$[M+H]^+$	255.065 2	255.064 7	199.075 0	BH	[18]
21.98	Licochalcone B	$C_{16}H_{14}O_5$	$[M-H]^-$	285.076 8	285.077 2	121.064 5	GC	[27]
22.48	Acacetin	$C_{16}H_{12}O_5$	$[M+H]^+$	285.075 8	285.075 2	270.051 6	BH	[18]
22.77	Daidzin	$C_{21}H_{20}O_9$	$[M+H]^+$	417.118 0	417.119 7	297.078 0	ZYCH	[28]
22.79	Neoliquiritin	$C_{21}H_{22}O_9$	$[M+H]^+$	419.133 7	419.134 5	257.080 1, 137.023 1	GC	[29]
23.11	Isoliquiritigenin	$C_{15}H_{12}O_4$	$[M-H]^-$	255.066 3	255.066 7	135.008 9, 119.049 1, 91.054 7	GC	[27]
23.47	Butenophthalide	$C_{12}H_{12}O_2$	$[M+H]^+$	189.091 0	189.090 2	105.070 0	DG	[9]
24.21	Lactiflorin	$C_{23}H_{26}O_{10}$	$[M+HCOO]^-$	507.150 8	507.151 6	339.107 8, 177.055 6	BS	[13]
31.16	Genkwanin	$C_{16}H_{12}O_5$	$[M-H]^-$	283.061 2	283.061 4	268.037 9, 239.036 0, 211.039 7, 135.008 8	GC	[27]
31.28	Aloe emodin	$C_{15}H_{10}O_5$	$[M+H]^+$	271.060 1	271.059 4	215.069 6, 153.018 1	BH	[18]
33.81	Mudanpioside F	$C_{16}H_{24}O_8$	$[M+HCOO]^-$	389.145 3	389.158 0	181.085 5, 151.038 7	BS	[30]
36.38	Tricoumaroyl spermidine	$C_{34}H_{37}N_3O_6$	$[M+H]^+$	584.275 5	584.270 3	438.237 8, 420.228 5, 275.174 8	HH	[25]
36.39	Mudanpioside B	$C_{31}H_{34}O_{14}$	$[M-H]^-$	629.187 6	629.184 4	583.182 6, 121.029 5	BS	[11]
36.72	Poricoic acid B	$C_{30}H_{44}O_5$	$[M+H]^+$	485.326 2	485.325 1	467.314 5	FL	[31]
37.34	Dehydropachymic acid	$C_{33}H_{50}O_5$	$[M+H]^+$	527.373 1	527.335 8	509.325 9, 467.316 7	FL	[31]
40.58	Mudanpioside J	$C_{31}H_{34}O_{14}$	$[M-H]^-$	629.187 6	629.188 0	121.029 5	BS	[11]
40.69	Dehydroeburicoic acid	$C_{31}H_{48}O_3$	$[M+H]^+$	469.367 6	469.330 3	451.319 4	FL	[31]
42.03	Saikosaponin C	$C_{48}H_{78}O_{17}$	$[M+COOH]^-$	971.521 6	971.480 2	439.356 1, 421.345 6, 403.332 6, 309.257 2, 189.163 3	ZYCH	[32]
42.47	Saikosaponin F	$C_{48}H_{80}O_{17}$	$[M+COOH]^-$	973.537 2	973.534 2	927.534 2, 706.391 0, 585.416 6, 451.319 9, 423.360 6, 309.117 7, 201.162 8, 105.069 2	ZYCH	[32]
43.17	Senkyunolide A	$C_{12}H_{16}O_2$	$[M+H]^+$	193.122 3	193.122 0	175.111 3, 147.116 5, 137.059 5	DG	[9]
44.20	Dibutyl phthalate	$C_{16}H_{22}O_4$	$[M+H]^+$	279.159 1	279.158 3	205.158 4, 149.023 1, 131.048 8	BS	[8]
45.12	*Z*-Ligustilide	$C_{12}H_{14}O_2$	$[M+H]^+$	191.106 6	191.106 1	173.095 8, 145.100 9	DG	[33]
45.48	Glycycoumarin	$C_{21}H_{20}O_6$	$[M-H]^-$	367.118 7	367.118 9	309.109 5, 283.023 2, 135.043 9	GC	[27]
46.72	Saikosaponin D	$C_{42}H_{68}O_{13}$	$[M+COOH]^-$	825.456 4	825.446 8	779.460 0, 617.406 7	ZYCH	[32]
47.42	3β,16α-Dihydroxylanosta-7,9(11),24-trien-21-oic acid	$C_{30}H_{46}O_4$	$[M+H]^+$	471.346 9	471.346 1	317.210 3, 189.634 0, 121.101 1	FL	[27]
48.12	Dehydrotrametenolic acid	$C_{30}H_{46}O_3$	$[M+H]^+$	455.352 0	455.350 5	437.340 6	FL	[27]
48.19	Saikosaponin A	$C_{42}H_{68}O_{13}$	$[M+COOH]^-$	825.464 2	825.460 0	779.460 0, 617.406 9, 439.321 6	ZYCH	[32]
48.45	Oleanolic acid	$C_{30}H_{48}O_3$	$[M+H]^+$	457.367 6	457.366 8	439.356 4	BH	[18]

Continued

RT/min	Component	Formula	Formula	Calculated mass	Measured mass	MS/MS	Source	Reference
49.74	10,12-Octadecanedioic acid	$C_{18}H_{28}O_2$	$[M+H]^+$	277.216 2	277.215 6	259.204 9	DG	[9]
50.68	Saikosaponin B2	$C_{42}H_{68}O_{13}$	$[M+COOH]^-$	825.463 6	825.418 3	779.460 0, 617.404 0, 439.321 6	ZYCH	[32]
51.13	Glycyrrhizic acid	$C_{42}H_{62}O_{16}$	$[M-H]^-$	821.396 5	821.398 1	351.057 1, 193.035 4	GC	[27]
53.99	Licoricone	$C_{22}H_{22}O_6$	$[M-H]^-$	381.134 4	381.134 8	351.087 8, 323.056 4, 307.098 4, 201.019 7	GC	[27]
54.18	Glucyrin	$C_{22}H_{22}O_6$	$[M-H]^-$	381.134 4	381.135 0	351.087 7, 323.056 5, 279.030 8, 201.019 4, 149.061 1	GC	[27]
54.29	Licoisoflavanone	$C_{20}H_{18}O_6$	$[M-H]^-$	353.103 1	353.103 4	227.105 4, 165.017 4	GC	[27]
54.51	Atractylenolide I	$C_{15}H_{20}O_2$	$[M+H]^+$	233.153 6	233.153 1	215.142 6, 187.147 7, 131.085 4	BZ	[14]
55.10	Acetylsaikosaponin B_2 (6''-*O*-acetyl-saikosaponin B_2)	$C_{44}H_{70}O_{14}$	$[M+COOH]^-$	867.473 7	867.433 3	821.471 5, 779.459 2, 617.407 5	ZYCH	[32]
55.84	Acetylsaikosaponin D	$C_{44}H_{70}O_{14}$	$[M+COOH]^-$	867.473 7	867.470 5	779.462 1, 761.447 4	ZYCH	[32]
58.01	Pachymic acid	$C_{33}H_{52}O_5$	$[M+H]^+$	529.388 8	529.351 7	469.329 8	FL	[31]
58.85	Angelicide	$C_{24}H_{28}O_4$	$[M+H]^+$	381.206 6	381.204 7	363.194 8, 335.200 4, 191.106 3	DG	[9]
59.79	Atractylenolide II	$C_{15}H_{18}O_2$	$[M+H]^+$	231.138 0	231.137 9	213.126 8, 185.132 1, 157.101 0, 143.085 3, 129.069 5	BZ	[14]
62.70	Safflor yellow A	$C_{27}H_{30}O_{15}$	$[M+H]^+$	595.165 7	595.168 3	577.153 4, 449.123 4, 433.090 7	HH	[24]
64.20	Glycyrrhetinic acid	$C_{30}H_{46}O_4$	$[M-H]^-$	469.332 3	469.332 9	425.343 1, 409.311 6, 355.264 7	GC	[27]
65.44	Hederagenin	$C_{30}H_{48}O_4$	$[M-H]^-$	471.348 0	471.348 3	407.334 0	BS	[10]
65.95	Poricoic acid A	$C_{31}H_{46}O_5$	$[M-H]^-$	497.327 2	497.328 1	425.304 7, 423.291 0	FL	[27]
66.55	Poricoic acid C	$C_{31}H_{46}O_4$	$[M-H]^-$	481.332 3	481.332 9	421.311 2	FL	[27]

2 疏肝解郁组潜在药效环节分析

疏肝解郁组中, 皂苷类成分来自君药竹叶柴胡, 少数蒽醌、有机酸类成分来自薄荷, 黄酮类成分二者兼有。建立上述成分候选靶标互作网络, 计算网络拓扑值后, 获得关键基因60个。功能挖掘结果显示 (图1), 柴胡中皂苷类成分候选靶标作用于胆汁、胰液等“疏泄”关联的体液分泌过程、氧化应激与血脂关联的肝脏疾病、炎症以及多种神经递质分泌与传导相关通路, 与柴胡疏肝解郁、解热的传统功效相符。黄酮及其苷类成分参与炎症和性激素调节相关过程。薄荷中的蒽醌及有机酸类成分作用于改善代谢、纠正“免疫–炎症”失衡、调节性激素及神经系统信号相关通路, 薄荷疏散风热, 作为使药可助柴胡疏散条达, 引表药入营卫以疏结滞之气[6], 而发挥襄助竹叶柴胡之功。

3 活血调经组潜在药效环节分析

根据网络拓扑值计算结果筛选获得活血调经组中药候选靶标集中关键基因共145个, 成分群富集通路与药理作用见图2。活血调经组中药主要含有萜类、酚酸类、苯酞类、黄酮类、醌式查尔酮和氨基酸等类型化合物, 其中萜类为单萜 (苷) 类成分, 如白芍中的芍药苷、氧化芍药苷、芍药内酯C、没食子酰芍药苷等, 以及三萜类成分常春藤皂苷元, 其对应的候选靶标主要作用于血液循环和性激素调节相关通路, 具有改善血液流变学、调节生殖功能、性激素分泌及血管生成等作用, 从而发挥养血柔肝、调经止痛之功。黄酮类成分群种类与疏肝解郁组所含类似, 其候选靶标亦在炎症以及性激素调节相关通路富集, 通过抗炎及雌激素样作用发挥调经止痛功效。苯酞类成分来源于当归, 同时也是当归挥发油的主要成分, 其对应候选靶标显著富集于炎症及神经系统调节相关通路, 药理研究[34]表明当归挥发油具有缓解神经源性和炎症性疼痛作用, 且能够抑制自发周期性的、前列腺素/乙酰胆碱氯化物诱导的、催产素诱导的多种子宫平滑肌收缩而缓解痛经, 从而发挥调经止痛之功。未作具体种类划分中的醌式查尔酮羟基红花黄色素A、红花黄色素A与亚精胺类成分三香豆素亚精胺来源于红花, 氨基酸类成分苯丙氨酸与精氨酸来源于当归, 其候选靶标富集于性激素调节与血液循环关联通路, 发挥活血通经与调经止痛功效。酚酸类成分主要来源于当归、红花和白芍, 作用于性激素、物质以及激素代谢相关通路, 发挥活血、调经、柔肝功效。

4 益气健脾组潜在药效环节分析

益气健脾组中药所含化学成分包括萜类、黄酮类、香豆素类、三萜皂苷及氨基酸类化合物, 其中萜类主要为三萜类成分, 包括茯苓中的茯苓酸、松苓新酸等, 甘草中的甘草次酸, 以及倍半萜类成分白术内酯。根据网络拓扑值计算结果筛选获得关键基因138个。网络分析结果显示 (图3), 萜类成分群候选靶标主要富集于激素调节、血液循环系统、消化系统、免疫系统相关通路, 发挥调节代谢、增强免疫、改善血液循环等药理作用, 体现其益气调血、利水渗湿功效。黄酮类成分来源于甘草, 其对应的候选靶标富集于性激素、甲状腺激素

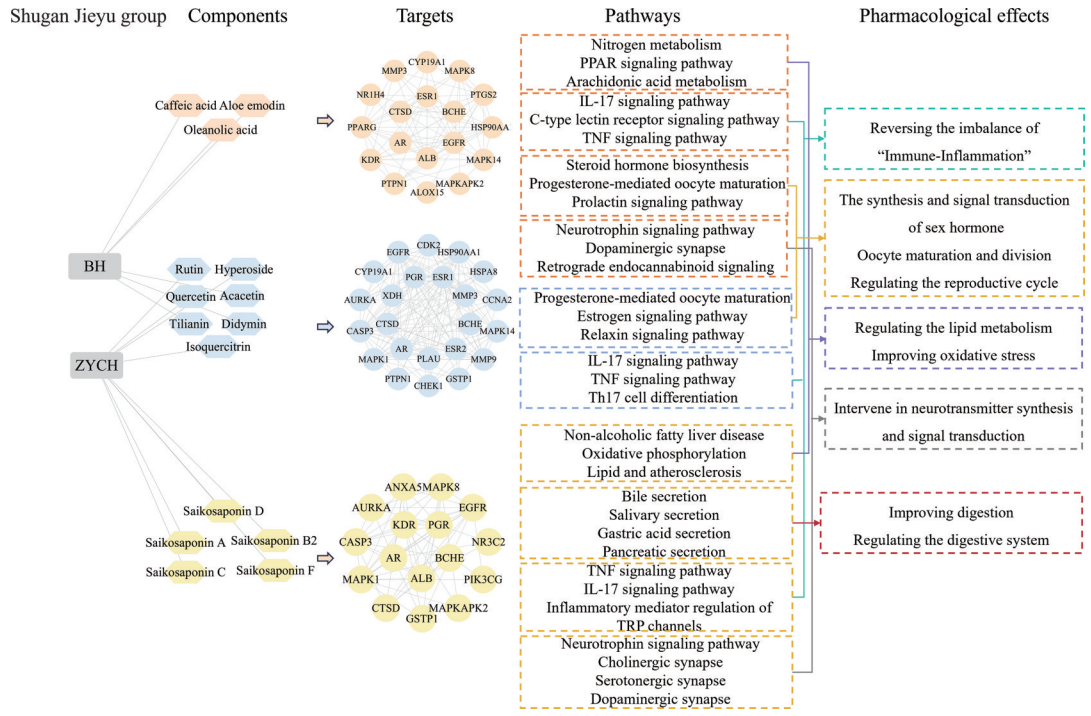

Figure 1 The mining of biological connotation in the Shugan Jieyu group. Rectangular targets represent herbs, hexagonal targets represent chemical components, circular targets represent core genes. Blue targets represent flavonoids (glycosides) and their corresponding targets, yellow targets represent saponins (glycosides) and their corresponding targets, and orange targets represent a small number of unclassified components and their corresponding targets. Same as in the Figures 2 and 3

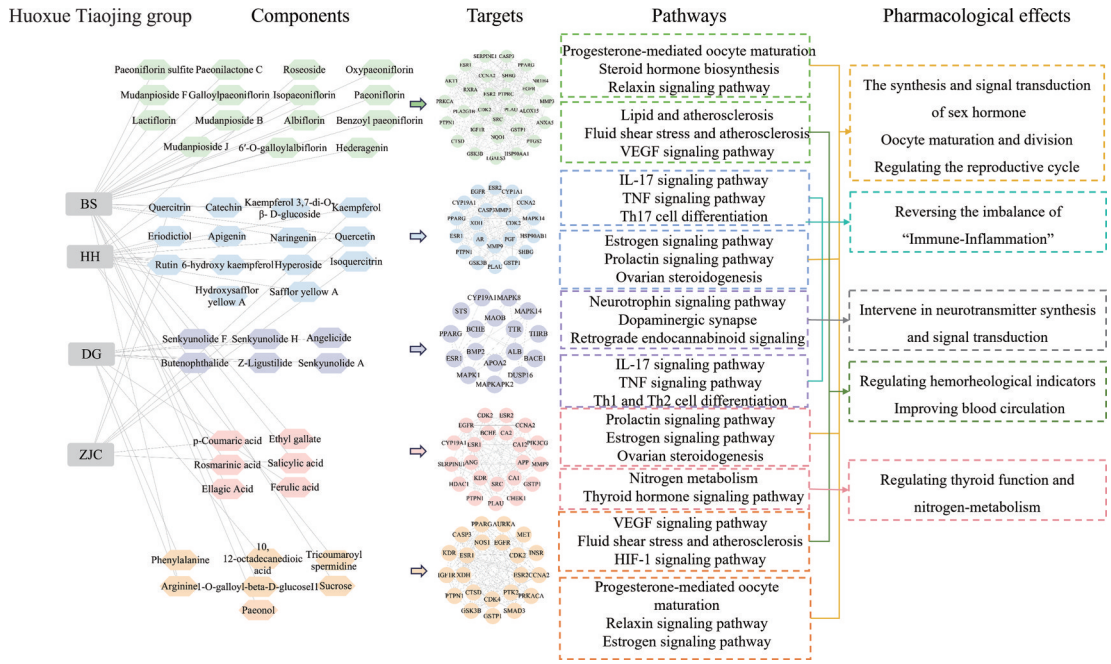

Figure 2 The mining of biological connotation in the Huoxue Tiaojing group. Green targets represent terpenoids and their corresponding targets, purple targets represent phthalide compounds and their corresponding targets, and pink targets phenolic acid components and their corresponding targets. Same as in the Figure 4

及醛固酮等多种激素相关通路, 发挥调经、益气等功效。香豆素类、氨基酸类、三萜皂苷类成分的靶标富集于纤凝系统、血流变调节关联的血液循环与炎症相关通路, 发挥活血、益气功效。

5 疏肝解郁组干预PMS的网络调控作用分析

构建"疏肝解郁组候选靶标-PMS肝郁气滞血瘀证

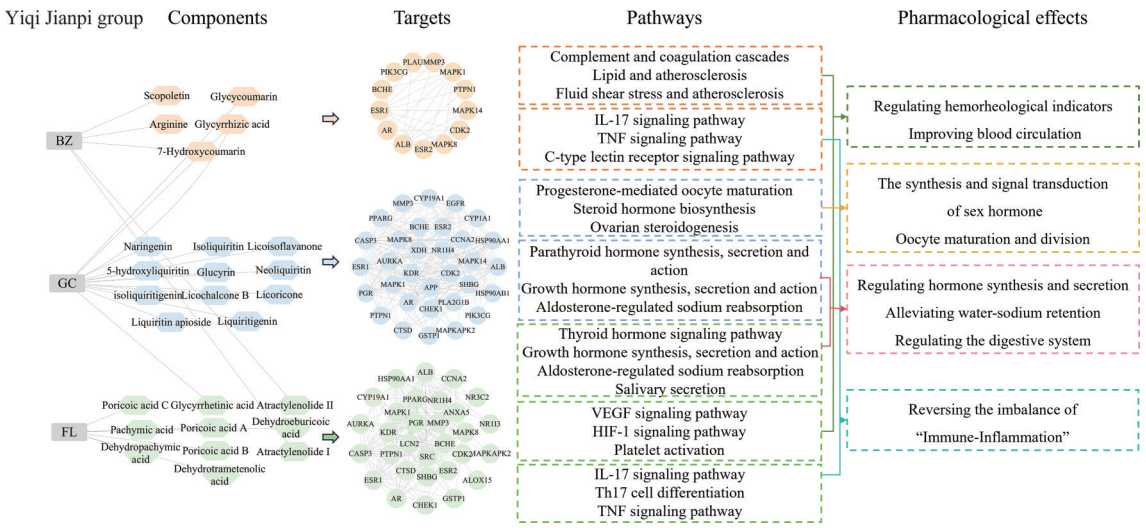

Figure 3 The mining of biological connotation in the Yiqi Jianpi group

相关基因”互作网络, 筛选212个连接度、介度和紧密度均在中位数以上的核心基因, 参与调节机体“内分泌–神经系统–免疫”相关通路 (图4)。疏肝解郁组主要作用于神经系统中神经营养素信号通路、血清素能突触、多巴胺能突触、神经活性配体–受体相互作用、胆碱能突触、逆行内源性大麻素信号传导、谷氨酸能突触等神经递质相关通路及疼痛相关的钙离子信号通路, 进而调节神经递质浓度和降低神经元兴奋性, 有助于改善PMS患者抑郁、烦躁、失眠等情志异常与痛经、胀痛等疼痛症状。同时作用于炎性介质调节以及炎症因子释放与聚集相关通路, 有助于改善PMS患者子宫炎症引起的痛经与神经炎症相关的头痛症状, 这一环节与薄荷襄助柴胡解热之功对应。疏肝解郁组能够作用于松弛素、雌激素、催产素、促性腺激素释放激素等性激素为主的内分泌系统信号通路, 与改善PMS患者的周期性出现的多种疾病症状有关。

6 活血调经组干预PMS的网络调控作用分析

构建“活血调经组候选靶标-PMS肝郁气滞血瘀证相关基因”互作网络 (图5), 筛选286个连接度、介度和紧密度均在中位数以上的核心基因, 主要参与直接调节血液循环系统过程, 如血液流变学相关通路 (脂质与动脉粥样硬化、流体剪切应力与动脉粥样硬化)、血管功能调节相关通路 (VEGF信号通路、血管平滑肌收缩、HIF-1信号通路), 以及血小板激活和纤凝系统相关通路 (血小板活化、补体和凝血级联反应)。同时, 参与多种性激素调节 (雌激素信号通路、松弛素信号通路、

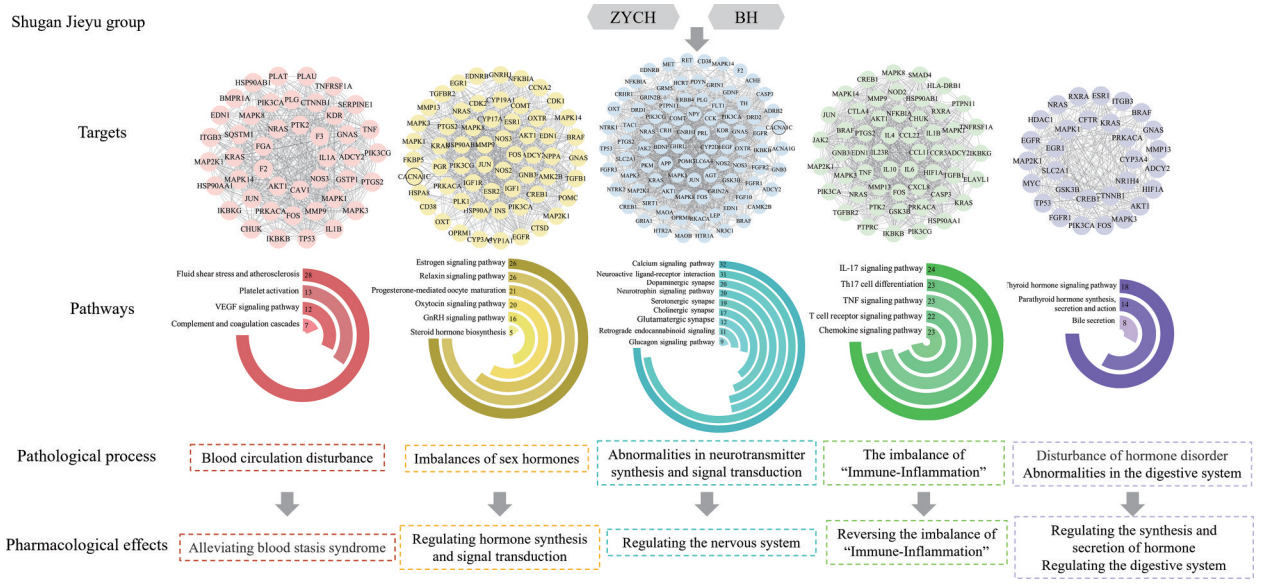

Figure 4 "Candidate targets of Shugan Jieyu group-PMS with liver depression, Qi stagnation and blood stasis syndrome genes" interaction network

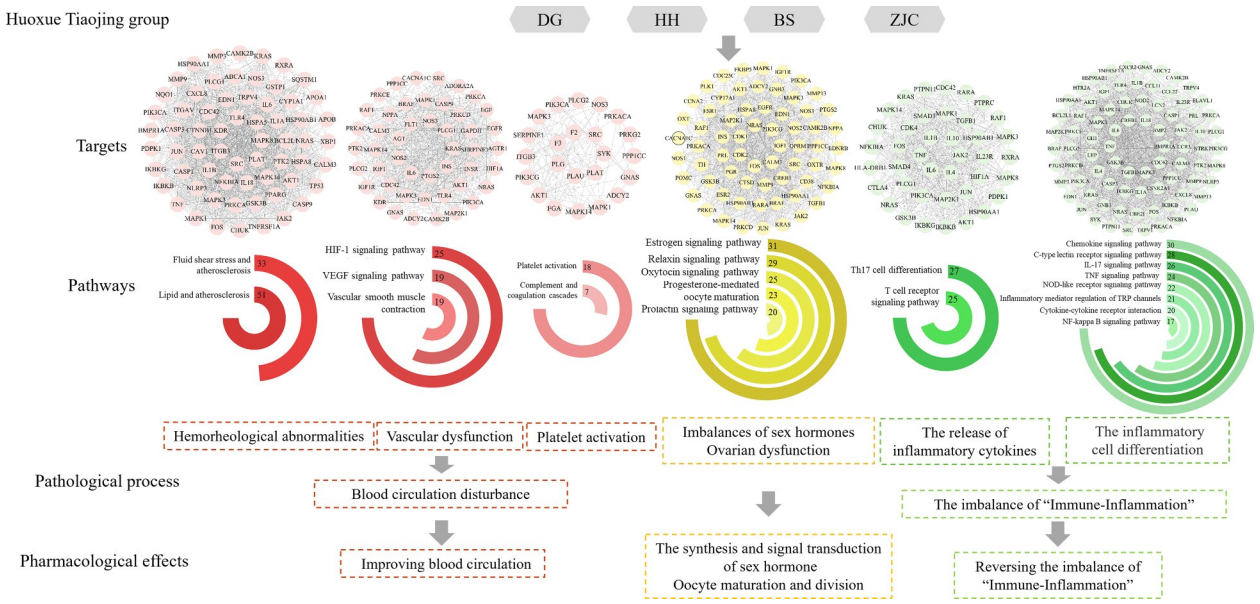

Figure 5 "Candidate targets of Huoxue Tiaojing group-PMS with liver depression, Qi stagnation and blood stasis syndrome genes" interaction network

催乳素信号通路和催产素信号通路) 与卵母细胞成熟相关通路 (孕酮介导的卵母细胞成熟), 以及多条“免疫−炎症”相关通路 (TNF 信号通路、IL-17 信号通路、趋化因子信号通路等)。

7 益气健脾组干预 PMS 的网络调控作用分析

构建“益气健脾组候选靶标-PMS 肝郁气滞血瘀证相关基因”互作网络 (图 6), 筛选 282 个连接度、介度和紧密度均在中位数以上的核心基因, 主要参与“免疫−炎症”相关通路, 如趋化因子信号通路、IL-17 信号通路、C 型凝集素受体信号通路、Th17 细胞分化、T 细胞受体信号通路等。同时参与生长激素、甲状腺素、醛固酮及肾素等激素水平调节, 改善内分泌系统功能, 有助于缓解 PMS 中烦躁以及水钠潴留引起的肿胀症状。此外, 益气健脾组能够作用于唾液、胆汁和胃酸分泌等过程进而改善消化系统功能。

8 红花逍遥片缓解 PMS 的药效学评价

8.1 红花逍遥片可有效纠正 PMS 大鼠性激素水平紊乱 由图 7 可见, 与溶剂对照组相比, PMS 模型组大鼠

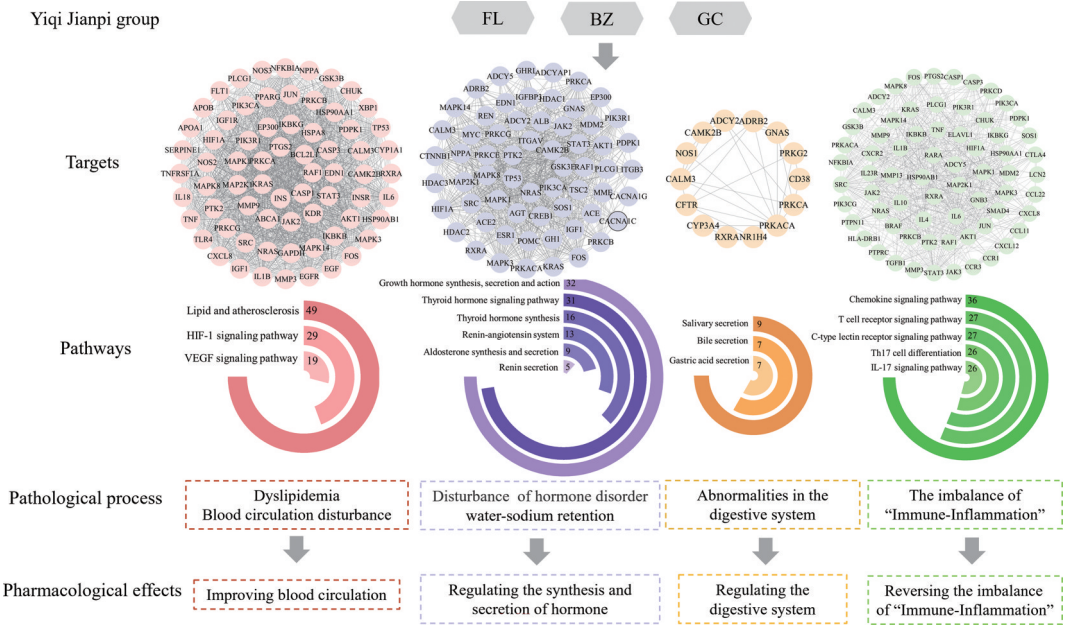

Figure 6 "Candidate targets of Yiqi Jianpi group-PMS with liver depression, Qi stagnation and blood stasis syndrome genes" interaction network

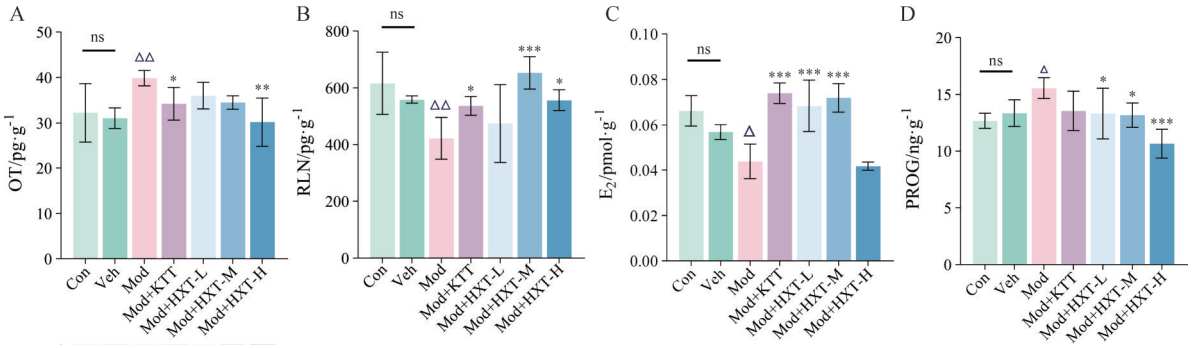

Figure 7 The effects of HXT in regulating sex hormone levels. A–D: The level of oxytocin (OT, A), relaxin (RLN, B), estradiol (E_2, C) and progesterone (PROG, D) in the uterus. $n = 4$, $\bar{x} \pm s$ (ELISA). $^{\triangle}P < 0.05$, $^{\triangle\triangle}P < 0.01$ *vs* Veh; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *vs* Mod. Con: Control group; Veh: Vehicle group; Mod: Model group; Mod+KTT: Model+positive drug group; Mod+HXT-L: Model+low-dose HXT group; Mod+HXT-M: Model+medium-dose HXT group; Mod+HXT-H: Model+high-dose HXT group

子宫组织性激素PROG、OT水平显著上升 ($P < 0.05$, $P < 0.01$), E_2、RLN水平显著降低 ($P < 0.05$, $P < 0.01$); 与PMS模型组相比, 红花逍遥片低、中、高剂量可不同程度下调PROG水平 ($P < 0.05$, $P < 0.001$), 高剂量组OT水平显著降低 ($P < 0.01$), 低、中剂量组子宫组织E_2显著上调 ($P < 0.001$), 中、高剂量组RLN显著上升 ($P < 0.001$, $P < 0.05$)。

8.2 红花逍遥片可有效纠正PMS大鼠神经递质水平紊乱 由图8可见, 与溶剂对照组相比, PMS模型组大鼠下丘脑组织神经递质5-HT和DA水平显著降低 ($P < 0.05$, $P < 0.01$); 与PMS模型组相比, 中剂量红花逍遥片能够上调5-HT水平, 低、中剂量红花逍遥片能够不同程度上调DA水平 ($P < 0.05$, $P < 0.01$)。

8.3 红花逍遥片可有效降低PMS大鼠炎症因子水平 与溶剂对照组相比, PMS模型组大鼠子宫IL-6、IL-1β和TNF-α水平显著上升 ($P < 0.05$, $P < 0.001$); 与PMS模型组相比, 低、中、高剂量红花逍遥片给药可不同程度降低子宫组织多种炎症因子水平 ($P < 0.05$) (图9)。

8.4 红花逍遥片可有效改善PMS大鼠血液循环相关指标水平 与溶剂对照组相比, PMS模型组大鼠子宫组织ET-1和VEGF水平显著上升 ($P < 0.01$, $P < 0.001$), NO水平呈下降趋势 ($P < 0.01$); 与PMS模型组相比, 红花逍遥片低、中、高剂量组不同程度上调NO水平 ($P < 0.05$), 下调VEGF水平 ($P < 0.01$), 中、高剂量组显著降低ET-1水平 ($P < 0.01$)。综上, 红花逍遥片能够调节PMS病理状态下的生殖器官血液循环相关指标 (图10), 进而有效改善血瘀、痛经、经行不畅等症状。

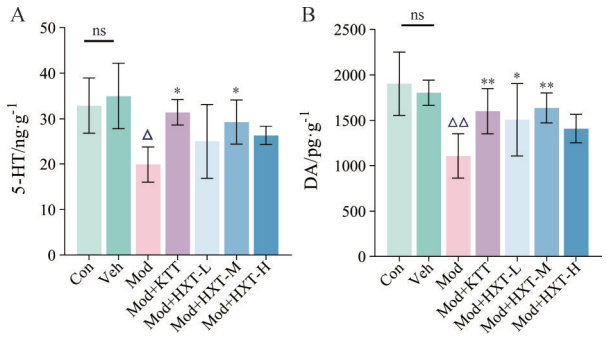

Figure 8 The effects of HXT in regulating neurotransmitter levels. A, B: The level of 5-hydroxytryptamine (5-HT, A) and dopamine (DA, B) in the hypothalamus. $n = 4$, $\bar{x} \pm s$ (ELISA). $^{\triangle}P < 0.05$, $^{\triangle\triangle}P < 0.01$ *vs* Veh; $^{*}P < 0.05$, $^{**}P < 0.01$ *vs* Mod

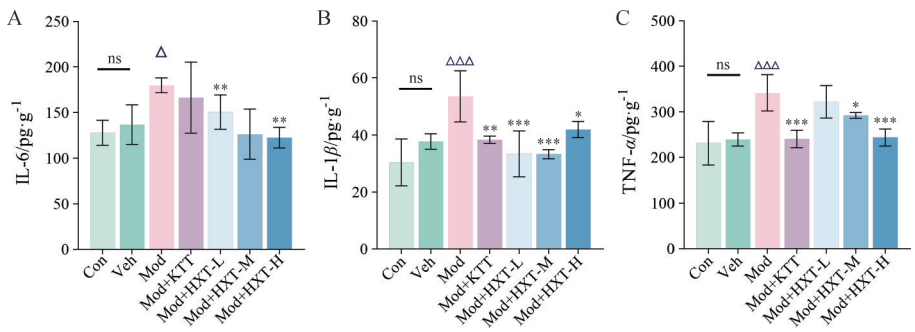

Figure 9 The effects of HXT in inhibiting inflammation. A–C: The level of interleukin-6 (IL-6), interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) in the uterus. $n = 4$, $\bar{x} \pm s$ (ELISA). $^{\triangle}P < 0.05$, $^{\triangle\triangle\triangle}P < 0.001$ *vs* Veh; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *vs* Mod

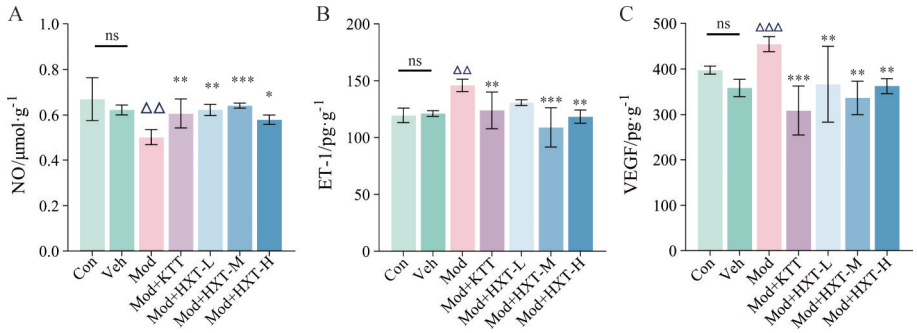

Figure 10 The effects of HXT in regulating blood circulation. The level of NO (A), endothelin-1 (ET-1, B) and vascular endothelial growth factor (VEGF, C) in the uterus. $n = 4$, $\bar{x} \pm s$ (ELISA). $^{\triangle\triangle}P < 0.01$, $^{\triangle\triangle\triangle}P < 0.001$ *vs* Veh; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *vs* Mod

讨论

本研究通过采用 UHPLC-Q Exactive Orbitrap HRMS 技术, 根据化合物的保留时间、分子式、精确相对质量和碎片离子等信息和相关文献, 系统地鉴定并推测出红花逍遥片中 109 个化学成分, 并对其进行饮片归属与成分归类。基于化学成分鉴定结果以及功效组划分建立了红花逍遥片疏肝解郁组、活血调经组与益气健脾组中药所含各类成分的候选靶标集, 网络挖掘结果表明各组能够针对内分泌、神经、免疫及血液循环等多个 PMS 肝郁气滞血瘀证关联的药效环节发挥协同作用, 提示其具有治疗 PMS 肝郁气滞血瘀证的潜力, 并进一步建立各功效组“病−证−症−方”互作网络深入挖掘红花逍遥片对证治疗 PMS 的生物学内涵与作用特点。

红花逍遥片所含成分主要为三萜皂苷类、萜类、黄酮类、酚酸类、苯酞类及少量其他种类。皂苷类成分主要来自柴胡, 柴胡为疏肝解郁要药, 具有解表退热、疏肝解郁、升举清气之功效, 其现代科学内涵可概述为一方面调节肝脏能量物质的代谢和转运、实体能量物质的储存以及肝生物学结构基础的保障, 另一方面缓解情志异常, 改善抑郁、焦虑等症状, 后者为 PMS 主要临床症状。柴胡皂苷 (saikosaponins, SS) 是柴胡的主要化学和生物活性成分[35], 其中尤以柴胡皂苷 A 和柴胡皂苷 D 药理活性最强[36]。药理实验表明, 柴胡皂苷 A 能够通过激活 tet1/dll3/notch1 信号传导并促进海马神经发生以改善小鼠的抑郁样行为[37], 柴胡皂苷 D 能够降低海马 CA1 区的谷氨酸水平, 并通过调节 Homer1-mGluR5 和下游 mTOR 信号通路促进突触蛋白 PSD-95 和 SYP 的表达预防抑郁症[38], 并能激活 ERβ 通路负调控 ROS/NLRP3 炎症小体来缓解肝脏炎症[39]。SS 对中枢神经系统功能的直接调节和抗炎作用与其疏肝解郁和解热的传统功效关联[40], 提示主要来自竹叶柴胡的皂苷类成分在 PMS 肝郁气滞血瘀证治疗中, 能够从缓解神经和肝脏炎症、调节神经递质分泌与信号传导、神经系统发育等角度多层次综合发挥调控“肝郁气滞”的药理作用。

女性月经、孕、产等生理环节受“肾气−天癸−冲任−胞宫”机制调节[41], 与西医学的“下丘脑−垂体−卵巢−子宫”的作用环路相对应[42]。从中医理论来看, 肾藏精, 主生殖, 雌激素相关功能与中医学“肾”的相关功能密切关联[43], 肾为天癸之源, 冲任之本, 气血之根, 与胞宫相连, 故有“经本出于肾”之说。肝肾之间, 肝之疏泄调度肾的闭藏, 使之开合有度, 肾之闭藏又制约肝脏, 使之疏泄有节。若肾精不足, 水不涵木, 肝失所养, 每遇情志不舒, 肝郁化火, 上扰心神而致 PMS 头痛等症[44]。除相对黄体功能不全、“子宫−脑轴”失衡、性激素水平的波动外, 有研究表明炎症反应、氧化应激也是 PMS 的病因之一, 即 PMS 通常伴有抗氧化状态下降和炎症潜能增加, 而胃肠道异常反应、各种疼痛与神经系统异常等相关症状便是与炎症和抗氧化生物标志物密切相关[45,46]。

黄酮类成分在红花逍遥片组方药物中广泛分布, 其中, 槲皮素、芦丁、异槲皮素等在 3 个功效组中均存在, 此类成分具有抗炎[47]与雌激素样作用, 研究表明其能提高雌性大鼠雌二醇、孕酮水平, 促进催乳素、生长激素分泌, 调节雌激素受体及其下游信号通路等[48], 因此有利于调节 PMS 肝郁气滞血瘀证中的性激素波动。薄荷中黄酮类成分具有显著抗炎以及调节雌激素作用: 一方面, didymin 通过抑制 TLR4/NF-κB 通路的激活, 调节 PLA2G4B、LPCAT3 和 CEPT1 的表达来抑制甘油磷脂代谢途径, 从而减轻代谢紊乱和肝细胞损伤, 显著减少促炎细胞因子的释放, 减轻炎症损伤[49,50], 发挥保肝作用; 另一方面, acacetin 能够恢复卵巢切除小鼠的雌激素水平, 发挥雌激素样作用[50]。薄荷作为佐使药, 可助柴胡疏散条达, 顺肝木之性, 为肝之所喜, 共奏清肝达郁之效[6]。甘草中的黄酮类成分为益气健脾组独有, 与 PMS 中的甲状腺激素、醛固酮等内分泌系统激素调节有关[51], 在改善情绪异常与改善水钠潴留

等症状中发挥作用。红花中醌式查尔酮类成分为特殊的黄酮类成分, 红花作为妇科疾病常用药, 主要活性成分羟基红花黄色素A与内源性雌激素结构和功能类似[52], 能够作用于ERα与ERβ进而发挥雌激素样作用, 经典的雌激素靶组织包括中枢神经系统、乳腺、子宫、肝脏、心血管系统等[53], 黄体期发生的多种雌激素水平变化导致相应的靶组织出现异常, 因此推测雌激素相关受体的广泛分布可能是多脏腑失和诱发PMS症状的原因之一。综上可知, 红花逍遥片中的黄酮类成分具有雌激素样作用与抗炎作用, 能够通过内分泌与免疫系统的调节发挥调经、解郁、益气功效, 并协同君药竹叶柴胡疏肝功效。

PMS肝郁气滞血瘀证发病机制以肝疏泄不及为主, 伴随肾、脾、心等脏腑失和, 以及伤及气血, 致其失调。女性月事、孕育、生产、哺乳等生理过程以血为用, 多因经、孕、产、乳以及情志等数伤于血[54]。同时, 脾虚是PMS发展中的环节, 经血生化源于脾, 脾虚则伤及元气, 与肝郁同作, 形成虚气留滞动态变化, 元气亏虚, 气血阴阳受损; 虚则加重留滞, 形成瘀血、火热和浊毒等壅聚[44], 故PMS患者出现情志异常等主要症状的同时, 伴随诸多复杂的病理状态, 如食欲不振、痛经、乳房胀痛、小腹胀痛、水肿等。萜类成分主要来自白芍与益气健脾组中药, 与调经柔肝、调和气血、益气健脾等功效关联, 有助于缓解上述临床症状。网络分析结果表明, 白芍中的单萜及其苷类成分通过调节血流变、脂质代谢以及性激素发挥养血调经功效。此外, 白芍苷类成分能够通过调节肠道微生物群组成来促进柴胡皂苷的转化以减弱其对谷胱甘肽合成酶活性的抑制作用, 从而预防柴胡皂苷类成分诱导氧化应激与炎症介导的肝毒性[55], 使柴胡“散而不伤肝阴”, 养肝体助肝用, 与白芍柔肝敛阴功效关联。白术内酯I与白术内酯III为白术倍半萜类成分中的主要活性成分, 通过调节氧化应激、减轻炎症反应、激活抗凋亡信号通路和抑制细胞凋亡发挥抗炎和器官保护特性, 这些保护作用延伸到心、肝、肺、肾、胃、肠和神经系统, TLR4/NF-κB、PI3K/Akt和MAPK信号通路主要介导这类化合物的抗炎作用[56], 并有助于缓解PMS中神经系统异常导致的抑郁情绪。茯苓与甘草含有三萜酸类成分。茯苓中的茯苓酸、去氢茯苓酸、茯苓新酸B等四环三萜类化合物是茯苓利尿的主要有效成分[57], 其作用与“肾素–血管紧张素–醛固酮”系统功能调节有关。茯苓中三萜类成分具有潜在的拮抗醛固酮受体活性, 通过激活肾组织Na^+-K^+-ATP酶, 能促进机体水盐代谢[58], 通过利尿消肿改善肾阳虚水肿, 利水除湿以防水湿凌心, 健脾以养心气宁心[59]。红花逍遥片中的萜类成分通过纠正免疫失衡、调节激素水平以及消化功能以扶正固本、益气健脾、利水渗湿, 有助于改善PMS中情志异常、食欲不振、胀痛、水肿等症状。

红花逍遥片中的酚酸类成分来自薄荷及活血调经组中药, 其中主要以阿魏酸、水杨酸、对香豆酸、咖啡酸等小分子酚酸为主, 此类化合物结构中苯环上通常存在多个酚羟基取代, 具有抗炎、抗氧化活性。已有研究表明, 薄荷所含的咖啡酸主要通过抑制MAPK和NF-κB等信号通路的活化, 抑制促炎性细胞因子TNF-α、IL-1β、IL-6的分泌, 从而发挥抗炎作用, 具有显著神经保护作用, 减轻神经为主炎症反应与氧化应激损伤[60]。活血调经组的酚酸类成分阿魏酸能够抑制氧化应激, 改善卵母细胞质量, 促进其发育与成熟[61,62]。对香豆酸、迷迭香酸能够减轻LPS诱导的神经炎症, 减少IL-1β的释放, 改善脂质过氧化发挥抗抑郁作用[63,64]。多种酚酸类成分有利于改善神经与肝脏中的氧化应激与炎症反应, 发挥柔肝活血作用, 襄助柴胡疏肝解郁之功, 有利于缓解PMS伴随的情绪异常、全身炎症及血液循环障碍等症状。

藁本内酯、丁烯基苯酞、洋川芎内酯等苯酞类成分为当归挥发油的主要成分, 具有调节神经递质和改善炎症等神经保护作用。近年当归苯酞类成分的神经系统药理作用相关研究逐渐增加, 其与改善突触后膜电位、减轻突触和神经元结构的异常改变以及增加BDNF有关[65]。有研究发现, 当归活性成分藁本内酯可降低全血黏度、改善血流情况, 改善抑郁动物模型大脑内神经损伤, 进而缓解抑郁[66], 将其神经功能关联的抗抑郁功能与传统功效中活血止痛作用建立了联系。作为当归最主要的脂溶性有效组分, 苯酞类成分具有神经保护及增强药物在大脑吸收等药理活性, 进而有利于改善PMS发生时眩晕心悸、经闭痛经、月经不调、肠燥便秘等症状, 发挥当归补血活血、调经止痛、润肠通便之功。

综上, 本研究基于液质联用技术鉴定了红花逍遥片所含的化学成分, 通过构建“病–证–症–方”关联网络分析, 初步揭示了红花逍遥片中疏肝解郁组、活血调经组和益气健脾组3个功效组下各成分群在PMS肝郁气滞血瘀证治疗中的作用特点和生物内涵。发现该方通过皂苷类与苯酞类成分主要调节单胺类神经递质系统、调节内分泌系统、调节血液循环、神经可塑性、神经炎症、抗氧化应激发挥抗抑郁作用; 通过黄酮类成分主要调节雌二醇、孕激素等性激素水平与生殖细胞分化等生殖系统功能; 通过酚酸类、萜类成分矫正“免疫–炎症”失衡, 整方疏肝解郁、活血通经、益气健脾, 标本同治, 多途径、多靶点干预PMS肝郁气滞血瘀证中的

内分泌、血液循环系统和消化系统中的关键病理过程，并进一步通过构建PMS大鼠模型，对红花逍遥片在以上病理环节的干预作用进行验证，多个指标检测结果表明，该中成药品种具有调节机体“神经–内分泌–免疫”系统与血液循环系统的作用，缓解PMS中的脏腑失和与气血失调，为进一步展开红花逍遥片干预PMS肝郁气滞血瘀证的药效研究与机制探索奠定了良好的数据基础。

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