多维指标和生态因子关联分析揭示不同产区三七药材质量的关键驱动因子

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摘要: 三七药材质量影响其临床治疗效果。目前三七质量参差不齐, 其质量控制主要依赖于皂苷类化合物, 无法系统地反映三七药材品质。本研究基于不同产区三七药材的收集, 采用微量法、紫外−可见分光光度法、高效液相色谱法定量分析影响三七功效及剂型的多维度指标。结果表明, 三七中大分子物质 (淀粉、纤维素、木质素) 含量较高的产区为云南曲靖沾益炎方乡, 总多糖含量较高的产区为云南文山州麻栗坡麻栗镇, 总黄酮含量较高的产地为云南省玉溪华宁青龙镇, 三七素含量较高的产地为云南昆明寻甸甸沙乡, 总皂苷含量较高的产地为云南文山州麻栗坡麻栗镇。基于层次聚类和正交偏最小二乘法判别分析 (orthogonal partial least squares-discrimination analysis, OPLS-DA), 确定了三七素、总多糖、支链淀粉、纤维素可以作为区分不同产区三七药材的潜在生物标志物。利用药用植物全球产地生态适宜性区划信息系统获取三七产区的19个生态因子, 结合相关性分析确定了生态因子对三七各成分积累的影响。其中, 年降水量、等温性、平均月温差、最冷月份最低温度、最暖季度平均温度、最湿季度降水量以及最暖季度降水量是影响三七质量的关键生态因子。综上, 本研究全面评价了不同产地三七质量, 揭示了影响三七道地性品质的关键生态因子, 为后续三七质量控制和合理选址提供了参考依据。

关键词: 三七; 不同产区; 多维度指标含量; 多元统计分析; 生态因子

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Correlation analysis of multi-dimensional indicators and ecological factors reveals the key driving factors for the quality of *Panax notoginseng* in different origins

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Abstract: The quality of *Panax notoginseng* medicinal herbs affects their clinical treatment efficacy. At present, the quality of *Panax notoginseng* varies greatly, and its quality control mainly relies on saponin compounds, which cannot systematically reflect the quality of *Panax notoginseng* medicinal materials. This study is based on the collection of *Panax notoginseng* medicinal materials from different production areas, using micromethod, UV

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visible spectrophotometry, and high-performance liquid chromatography to quantitatively analyze multidimensional indicators that affect the efficacy and formulation of *Panax notoginseng*. The results showed that the production area with high content of macromolecules (amylum, cellulose, lignin) in *Panax notoginseng* was Yanfangxiang, Zhanyi, Qujing, Yunnan. The production area with high content of total polysaccharides was Malipo, Malizhen, Wenshan, Yunnan. The production area with high content of total flavonoids was Qinglongzhen, Huaning, Yuxi, Yunnan. And the production area with high content of *Panax notoginseng* was Dianshaxiang, Xundian, Kunming, Yunnan. The production area with high content of total saponins was Malipo, Malizhen, Wenshan, Yunnan. Based on hierarchical clustering and orthogonal partial least squares-discrimination analysis (OPLS-DA), dencichine, total polysaccharides, amylopectin, and cellulose were identified as potential biomarkers for distinguishing *Panax notoginseng* medicinal materials from different production areas. Using the global geographic information system for medicinal plants, 19 ecological factors of *Panax notoginseng* production areas were obtained, and the impact of ecological factors on the accumulation of various components of *Panax notoginseng* was determined through correlation analysis. Among them, the annual precipitation, the isothermality, the mean diurnal range, the min temperature of coldest month, the mean temperature of warmest quarter, the precipitation of wettest quarter, and the precipitation of warmest quarter were key ecological factors affecting the quality of *Panax notoginseng*. In summary, this study comprehensively evaluated the quality of *Panax notoginseng* from different origins, revealed the key ecological factors that affect its authentic quality, and provided a reference for subsequent quality control and rational site selection of *Panax notoginseng*.

Key words: *Panax notoginseng*; different production areas; multi-dimensional indicator content; multivariate statistical analysis; ecological factor

三七是五加科植物三七*Panax notoginseng* (Burk.) F. H. Chen的干燥根及根茎, 也是我国名贵的大宗药材, 具有散瘀止血、消肿定痛等功效[1], 广泛应用于心脑血管系统疾病、各种出血症的临床预防和治疗[2]。近年来, 对于三七的研究主要专注于品质评价[3,4]、指标分析[5,6]及生态因子[7,8]等多个方面, 这些研究成果的积累, 丰富了对三七生长特性和药效成分的深入理解, 为三七科学种植、有效提取及合理应用奠定了坚实的基础。三七中的有效成分主要为皂苷[9]、三七素[10]、多糖[11,12]、黄酮[13]等。此外, 还含有淀粉、木质素、纤维素等多种成分。三七总皂苷 (*Panax notoginseng* saponins) 具有降血脂、抗炎、抗氧化、抗肿瘤等药理作用, 其制剂常用于冠心病、高血压等多种心脑血管疾病的临床治疗[14]。其中, 三七皂苷主要分为原人参二醇型皂苷 (panaxadiol saponin, PPD) 和原人参三醇型皂苷 (panaxatriol saponin, PPT) 两大类[15]。PPD主要包括人参皂苷Rc (ginsenoside Rc)、人参皂苷Rb_1 (ginsenoside Rb1)、人参皂苷Rd (ginsenoside Rd) 等, 具有抗氧化[16]、抗肿瘤[17]和促进造血功能[18]等作用。PPT主要包括三七皂苷R_1 (notoginsenoside R1)、人参皂苷Rg_1 (ginsenoside Rg1)、人参皂苷Re (ginsenoside Re)、三七皂苷R_4 (notoginsenoside R4)、人参皂苷Rf (ginsenoside Rf) 和人参皂苷Rh_1 (ginsenoside Rh1) 等, 具有抗炎、免疫调节和促进造血功能等药理作用[19]。三七素 (dencichine) 属于非蛋白游离氨基酸, 是三七药材中止血功效的关键成分, 具有神经保护和抗炎等活性[20,21]。大量研究表明, 三七总多糖 (total polysaccharide) 具有抗肾纤维化、抗肿瘤、抗氧化等作用, 还可作为吸湿、保湿、载体和可溶解微针等材料, 具有重要的开发利用价值[22]。三七总黄酮 (general flavone) 作为主要成分之一, 普遍存在于花、果、叶等组织中, 具有抗菌、抗病毒等多种药理活性[23]。

大分子物质如淀粉、木质素和纤维素, 作为三七药材的重要物质基础, 影响三七药材质量, 尤其当三七作为全草入药的材料, 大分子物质是调节药物的物理性质和稳定性的关键成分。其中, 淀粉是植物中糖类的主要贮存形式, 包括支链淀粉 (amylopectin) 和直链淀粉 (amylose)[24]。中药材中大多含有淀粉, 何首乌和山药等部分根茎类药材淀粉含量极高[25]。现代研究表明, 淀粉基本无药理活性, 药材中淀粉含量过高可能会影响活性成份的积累, 使得有效成份含量降低, 引起药材及相关制剂服用剂量大、剂型开发困难等问题[26]。Wang等[27]认为甘薯的理化特性与口感品质有一定关联性, Liu等[28]研究发现淀粉与甘薯口感呈正相关关系。因此, 淀粉作为三七中的常见成分, 其含量与特性可影响药材的口感和稳定性, 进而影响药材的整体品质和临床应用效果。纤维素 (cellulose)[29]含量与特性同样影响着药材质量。一方面, 纤维素可以改善 消化系统中菌群微环境, 对人体的健康起着极为重要的作用[30], 适量纤维素含量可使药材口感更佳, 易于患者接

受; 另一方面, 纤维素形态特征同样是评估药材纯度、真实性及整体品质的重要依据[31]。木质素 (lignin) 广泛存在于木材和禾本科植物体内[32]。因其抗肿瘤、抗炎、抗病毒等活性协同其他药效成分影响着药材品质和治疗效果[33,34], 其独特的化学结构和生物活性也为鉴别药材真伪和品质优劣提供重要指标。综上, 大分子物质作为评价三七药材质量的物质基础, 具有重要的临床价值。然而, 目前针对不同产区三七淀粉、木质素、纤维素含量尚未进行系统研究。

中药材品质与其生长的地域环境密切相关[35], 这一观点早在《神农本草经校注》等古典医药著作中就有记载[36]。生态因子指环境中对生物的生长、发育、生殖、行为和分布等有着直接或间接影响的环境要素, 如光照、温度、水分和其他相关生物等[37]。现代研究表明, 药用植物区别于其他作物, 其经济价值不仅体现在产量, 更在于其品质 (即活性成分组成及含量) [38], 而环境因子是影响品质的关键因素, 药用植物体内生物碱、萜类、酚类和黄酮类等活性物质的形成和积累都与环境密切相关[39,40]。Meng 等[41]从品质生态学角度归纳阐述了环境因素对三七品质的影响, 为指导三七全球范围内生产基地合理布局、引种繁育等提供了依据。Mo 等[42]通过主成分分析、聚类分析有效区分了不同产地三七药材的质量存在差异, 说明三七质量受产区环境等因素影响。Ran 等[43]分析不同产地三七主根皂苷组分、总皂苷含量和 48 个生态因子的数量关系, 探明了影响三七主根皂苷组分的主导生态因子、主要决策因子和限制因子, 结果表明生态因子如温度、湿度与皂苷类物质含量具有相关性。然而生态因子与大分子物质、三七素、多糖、黄酮等多维度指标的相关性研究缺少系统性研究。通过药用植物全球产地生态适宜性区划信息系统 (global geographic information system for medicinal plant, GMPGIS) 分析三七多成分含量与生态因子的相关性, 确定影响三七成分含量的主要生态因子, 有助于指导三七种植的合理选址, 揭示三七道地性成因。

本研究以不同产地三年生三七为材料, 采用微量法、紫外−可见分光光度法、高效液相色谱法 (high-performance liquid chromatography, HPLC) 测定三七中淀粉、木质素、纤维素、总多糖、总黄酮、三七素以及多种皂苷的物质含量。基于含量比较、指纹图谱构建和多元统计分析, 明确了不同产地三七成分的差异, 确定区分不同产地三七药材质量的潜在标志物。进一步应用 GMPGIS 系统获取三七产区的 19 个生态因子, 通过分析生态因子与三七成分含量的相关性, 确定影响药材质量的关键生态因子, 初步揭示了影响三七道地性形成的环境因子, 从而为三七道地性评价和合理选址提供参考和科学依据。

材料与方法

仪器 高效液相色谱仪 (Agilent UPLC 1260 Infinity II, 美国 Agilent 公司), 多功能微孔板检测仪 (H1M, 美国伯腾仪器有限公司), 电热鼓风干燥箱 (DHG-9140A 型, 上海一恒科学仪器有限公司), 超声波清洗仪 (KQ-250E, 昆山市超声仪器有限公司), 电子天平 (ME203E/02, 梅特勒−托利多仪器上海有限公司), 多功能粉碎机 (800A, 浙江省永康市红太阳机电有限公司)。

对照品与试剂 *D*(+)-无水葡萄糖 (批号: 1122A0218), 购于北京索莱宝科技有限公司, 纯度, ≥ 98%; 芦丁 (批号: A05GB144263)、三七皂苷 R_1 (批号: M31HB180742)、人参皂苷 Rg_1 (批号: J08IB205578)、人参皂苷 Re (批号: A20HB192147)、三七皂苷 R_4 (批号: M31HB180742)、人参皂苷 Rf (批号: P25F12L140073)、人参皂苷 Rb_1 (批号: G01O11Y126429)、人参皂苷 Rc (批号: M27GB141849)、人参皂苷 Rh_1 (批号: M16J11S118466)、人参皂苷 Rd (批号: J03HB186914) 和三七素 (批号: R05N10F102244), 购于上海源叶生物科技有限公司, 纯度 ≥ 98%。支链淀粉含量检测试剂盒 (货号: BC4275)、直链淀粉含量检测试剂盒 (货号: BC4265)、木质素含量检测试剂盒 (货号: BC4205)、纤维素 (CLL) 含量检测试剂盒 (货号: BC4285), 购于北京索莱宝科技有限公司。浓硫酸、苯酚、浓硫酸、亚硝酸、硝酸铝、氢氧化钠、无水乙醇均为 AR 级; 乙腈、甲醇、磷酸均为 HPLC 级, 购于北京鑫葆海化学科技有限公司; 娃哈哈纯净水。

材料 在产区调研的基础上, 三七植株采挖于云南昆明、文山、玉溪、曲靖及贵州盘州、黔西南等 25 产地, 涵盖了三七的种植产区, 具有产区代表性。样品均为三年生三七, 其性状与现行《中华人民共和国药典》规定一致。各产区随机选取 30 个植株的根茎部作为样本, 每批样本经云南中医药大学徐福荣研究员鉴定均为三七 *Panax notoginseng*, 具体样品信息见表 1。

淀粉和木质素及纤维素含量测定 使用索莱宝支链淀粉含量检测试剂盒、直链淀粉含量检测试剂盒、木质素含量检测试剂盒、纤维素 (CLL) 含量检测试剂盒按照说明书分别检测三七中支链淀粉、直链淀粉、木质素、纤维素含量。

总多糖和总黄酮含量测定 根据文献[44,45], 制作总多糖、总黄酮含量标准曲线, 即总多糖标准曲线为 $Y = 4.873\ 3\ X + 0.003\ 7$, $R^2 = 0.996\ 2$; 总黄酮标准曲线

Table 1　*Panax notoginseng* sampling information

Origin number	Geographical situation	East longitude/°	North latitude/°	Altitude/m
C1	Xiaolongtan, Shilin, Kunming, Yunnan	103.419	24.903	1 886
C2	Walnut Garden, Shuanghe, Jinning, Kunming, Yunnan	102.431	24.523	2 212
C3	Long Ga, Ba Dao Sao, Qiubei, Wenshan, Yunnan	103.976	24.057	1 939
C4	Great Fire Zone, Qinglong, Chengjiang, Yuxi, Yunnan	102.931	24.465	2 114
C5	Longde, Santang, Luxi, Honghe,Yunnan	103.829	24.409	2 150
C6	Shayu , Huopu , Panzhou , Guizhou	104.422	25.611	2 125
C7	Ashahei, Mazheshao, Kaiyuan, Yunnan	103.354	23.714	2 131
C8	Qingxiangshu, Nijiao, Qiubei, Wenshan, Yunnan	103.906	23.901	1 694
C9	Xiaobantian, Guishan, Shilin, Kunming, Yunnan	103.575	24.720	1 991
C10	Wanmu Grassland, Qianxiang, Anlong, Xingyi, Qianxinan, Guizhou	105.460	25.212	1 363
C11	Shumidian, Longqing, Shizong, Qujing, Yunnan	104.076	24.682	2 057
C12	Zuozuobi, Nazhang, Malong, Qujing, Yunnan	103.654	25.288	2 137
C13	Daqinghe, Diansha, Xundian, Kunming, Yunnan	103.100	25.667	2 135
C14	Pipo, Mazheshao, Kaiyuan, Honghe, Yunnan	103.448	23.690	2 150
C15	Yiyi, Changhu, Shilin, Kunming, Yunnan	103.405	24.622	1 942
C16	Yanfang, Zhanyi, Qujing, Yunnan	103.998	26.002	2 090
C17	Xiaoshama, Xiangyang, Luxi, Honghe, Yunnan	103.900	24.527	2 071
C18	Shuomele, Xiangyang, Luxi, Honghe, Yunnan	103.921	24.544	2 021.5
C19	Shaguo, Huoshui, Luliang, Qujing, Yunnan	103.867	25.094	2 246.3
C20	Xinzhai, Qinglong, Huaning, Yuxi, Yunnan	102.938	24.334	2 027.3
C21	Shicha, Malipo, Wenshan, Yunnan	104.683	23.116	1 506
C22	Xialiangshuijing, Mali, Malipo, Wenshan, Yunnan	104.651	23.155	1 377
C23	Yandengjia, Mada, Malipo, Wenshan, Yunnan	104.634	23.111	1 592
C24	Panlong, Malipo, Wenshan, Yunnan	104.665	23.171	1 286
C25	Nanla, Daping, Malipo, Wenshan, Yunnan	104.597	23.074	1 347

为 $Y = 1.479\ 4\ X + 0.022\ 2$, $R^2 = 0.999\ 1$, 用于计算各样本总多糖、总黄酮含量。

三七素及皂苷含量的测定　对照品储备液制备: 取三七素对照品适量, 精密称定, 加30%甲醇分别制成每1 mL含对照品1 mg的溶液。使用30%甲醇稀释对照品浓度, 得到三七素最终质量浓度为0.10 mg·mL^{-1}, 用于确定保留时间并制作标准曲线 (表2)。

取三七皂苷R_1、R_4, 人参皂苷Rg_1、Rb_1、Re、Rd、Rf、Rc、Rh_1对照品适量, 精密称定, 加甲醇分别制成每1 mL含对照品1 mg的溶液。使用甲醇稀释对照品浓度, 得到三七皂苷R_1、人参皂苷Rg_1、Rb_1、Re、Rd最终质量浓度为0.50 mg·mL^{-1}, 三七皂苷R_4、人参皂苷Rf、Rc、Rh_1最终质量浓度为0.10 mg·mL^{-1}, 用于确定保留时间并制作标准曲线 (表2)。

Table 2　Reference standard curve. NG-R1: Notoginsenoside R1; G-Rg1: Ginsenoside Rg1; G-Re: Ginsenoside Re; NG-R4: Notoginsenoside R4; G-Rf: Ginsenoside Rf; G-Rb1: Ginsenoside Rb1; G-Rc: Ginsenoside Rc; G-Rh1: Ginsenoside Rh1; G-Rd: Ginsenoside Rd

Reference substance	Standard curve	R^2	Linear range/μg
Dencichine	$Y = 882.83\ X - 1.335\ 4$	1	0.025 8 − 0.600 3
NG-R1	$Y = 300.59\ X + 4.890\ 2$	0.999 9	0.241 5 − 7.515 2
G-Rg1	$Y = 346.11\ X - 4.314\ 5$	0.999 9	0.253 8 − 7.509 4
G-Re	$Y = 359.35\ X - 7.589\ 2$	0.999 9	0.261 6 − 7.509 5
NG-R4	$Y = 219.81\ X + 0.452\ 1$	1	0.050 4 − 1.504 4
G-Rf	$Y = 428.85\ X + 0.704\ 3$	0.999 9	0.048 8 − 1.503 3
G-Rb1	$Y = 246.89\ X + 4.509\ 8$	1	0.239 6 − 7.512 1
G-Rc	$Y = 298.06\ X + 0.913\ 2$	0.999 9	0.048 1 − 1.503 8
G-Rh1	$Y = 486.77\ X + 1.646\ 4$	0.999 9	0.047 9 − 1.503 6
G-Rd	$Y = 252.69\ X + 5.530\ 1$	1	0.238 1 − 7.498 9

供试品溶液制备: 取三七药材粉末0.1 g (过四号筛), 精密称定, 加入30%甲醇, 定容至20 mL, 涡旋振荡, 超声处理 (功率250 W, 频率40 kHz) 40 min, 放冷, 再次称定重量, 用30%甲醇补足减失的重量, 12 000 r·min^{-1}离心5 min, 上清液过0.22 μm有机滤膜, 保存于液相小瓶中 (4 ℃), 用于HPLC检测。

取三七药材粉末0.1 g (过四号筛), 精密称定, 加入甲醇, 定容至10 mL, 涡旋振荡, 超声处理 (功率250 W, 频率40 kHz) 40 min, 放冷, 再次称定重量, 用甲醇补足减失的重量, 12 000 r·min^{-1}离心5 min, 上清液过0.22 μm有机滤膜, 保存于液相小瓶中 (4 ℃), 用于HPLC检测。

三七素含量测定色谱条件: 参照文献[46], 对HPLC检测三七素方法进行优化。HILIC-Z色谱柱 (4.6 mm × 150 mm, 2.7 μm, Infinity-lab Poroshell 120 HILIC-Z, 美国Agilent公司), 柱温25 ℃, 流速1 mL·min^{-1}。流动相A为乙腈, B为0.1%磷酸水, 梯度洗脱, 0～15 min, 60%～15% A。检测波长220 nm, 进样量5 μL。

皂苷含量测定色谱条件: 参照《中华人民共和国药典》[1]中三七项下的高效液相色谱法进行优化。C18色谱柱 (4.6 mm × 250 mm, 5 μm, Eclipse XDB-C18, 美国

Agilent 公司), 柱温 25 ℃, 流速 1 mL·min^{-1}。流动相 A 为乙腈, B 为纯净水, 梯度洗脱, 0～40 min, 18%～21% A; 40～42 min, 21%～26% A; 42～46 min, 26%～32% A; 46～70 min, 32%～36% A。检测波长 203 nm, 进样量 10 μL。

19 个生态因子的获取 各产区生态因子来源于 World Clim 全球气候数据库 (World Clim-global climate data)[47]和 Cli Mond 全球生物气候学建模数据库 (Cli Mond: global climatologies for bioclimatic modelling)[48]。根据收集三七样品时所记录的经度、纬度及海拔信息, 通过 GMPGIS 得到不同产地三七的 19 个生态因子, 分别为年平均气温、平均月温差、等温性、气温季节性变动系数、最暖月份最高温度、最冷月份最低温度、气温年较差、最湿季度平均温度、最干季度平均温度、最暖季度平均温度、最冷季度平均温度、年降水量、最湿月份降水量、最干月份降水量、降水量季节性变化、最湿季度降水量、最干季度降水量、最暖季度降水量、最冷季度降水量。

数据分析 使用《中药色谱指纹图谱相似度评价系统》(2012 年版) 软件, 建立不同产地三七指纹图谱。通过小提琴图、柱状图对检测数据进行可视化, 并结合层次聚类分析 (hierarchical clustering analysis, HCA)、主成分分析 (principal component analysis, PCA)、OPLS-DA 等对其进行综合评价。聚类热图的聚类方法均采用 complete 法, 距离度量设置为 euclidean。HCA 的聚类方法均采用 ward.D2 法, 距离度量设置为 euclidean。HCA、PCA 使用 OmicStudio (https://www.omicstudio.cn/tool) 分析, OPLS-DA 使用 Metware Cloud (https://cloud.metware.cn) 进行分析。其余数据通过 Origin 2022 进行可视化处理, 使用 SPSS 进行单因素方差分析和 Tukey HSD (honestly significant difference) 检验。

结果与分析

1 不同产地三七淀粉和木质素及纤维素含量分析

不同产区三七中淀粉、木质素和纤维素含量存在差异, 支链淀粉含量范围在 11.905%～41.666%、直链淀粉含量范围在 2.070%～6.446%, 纤维素含量范围在 7.021%～39.774%, 木质素含量范围在 16.502%～48.715%, 密度范围分布离散 (图 1A)。

聚类分析表明, 25 份三七样品主要分为三簇, 其中大分子物质 (淀粉、木质素、纤维素) 含量较高的产区为云南曲靖沾益炎方乡, 主要分布在簇II (图 1B)。热图结果表明, 在各簇中支链淀粉与木质素含量较高, 直链淀粉含量较低, 纤维素则在簇I、II中含量较高, 簇III含量较低。在主成分分析中 (图 1C), 簇I和II样本间数据具有相似性, 在主成分空间分布上较为聚集, 簇III则与其他簇明显分离。综上, 纤维素可能是导致各样本大分子化合物差异的主要原因。

2 不同产地三七总多糖和总黄酮及三七素含量分析

不同产区三七中总多糖和总黄酮含量范围分别为

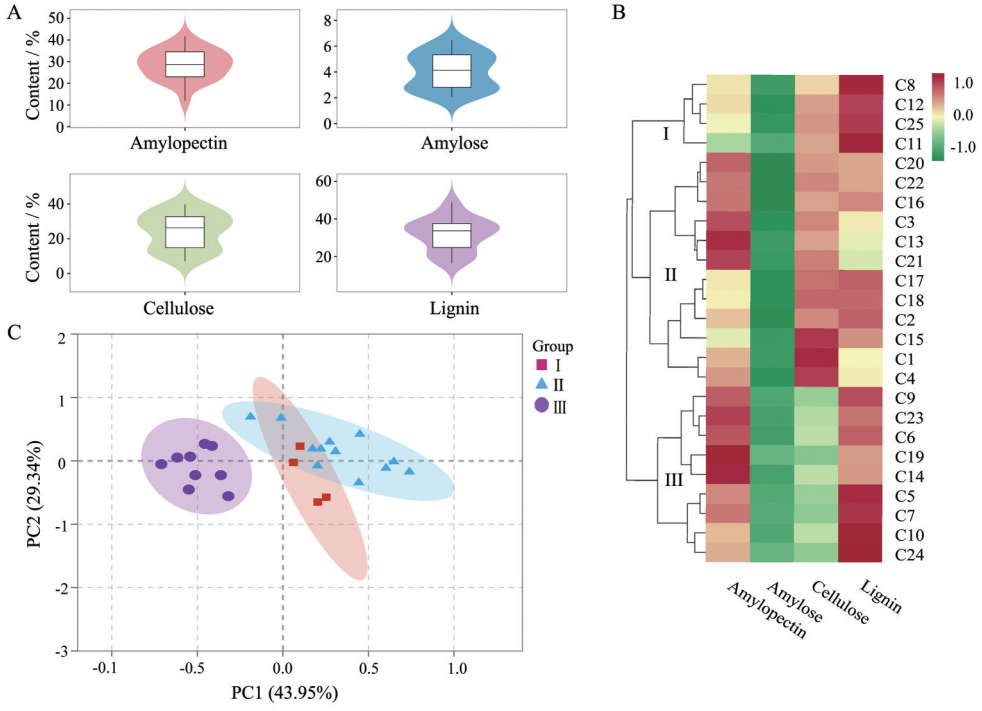


Figure 1 Analysis of macro-molecular content. A: Content distribution; B: Complex heatmap; C: Principal component analysis (PCA). C1−C25 are in accordance with those in Table 1. PC1: Principal component analysis 1; PC2: Principal component analysis 2

6.395%～25.475%和0.067%～0.233%, 二者含量分布均较为离散 (图2A)。其中, 三七总多糖的含量较高的产地为云南文山州麻栗坡麻栗镇盘龙村, 总黄酮含量较高的产地为云南省玉溪华宁青龙镇新寨村 (图2B)。聚类分析结果表明, 总多糖含量较高产区主要分布在簇I, 总黄酮含量较高产区主要在簇II中, 这说明了三七总多糖和总黄酮含量具有地域性 (图2C)。不同产地三七中三七素含量范围在0.467%～0.962% (图2D), 含量范围分布同样较为离散 (图2E), 其中含量较高的产区为云南昆明寻甸甸沙乡大清河村。

3　不同产地三七皂苷类物质含量分析

对25批三七样品的皂苷类物质进行检测, 得到HPLC色谱图。将色谱图导入《中药色谱指纹图谱相似度评价系统 (2012年版)》, 以C1作为参照图谱, 采用平均数法和多点校正, 时间宽度为0.1 min, 对色谱图进行匹配, 生成对照图谱R, 共得到13个共有峰。经对照品比对, 并根据保留时间进行定性分析, 共指认出9个共有色谱峰, 分别为三七皂苷R_1、人参皂苷Rg_1、人

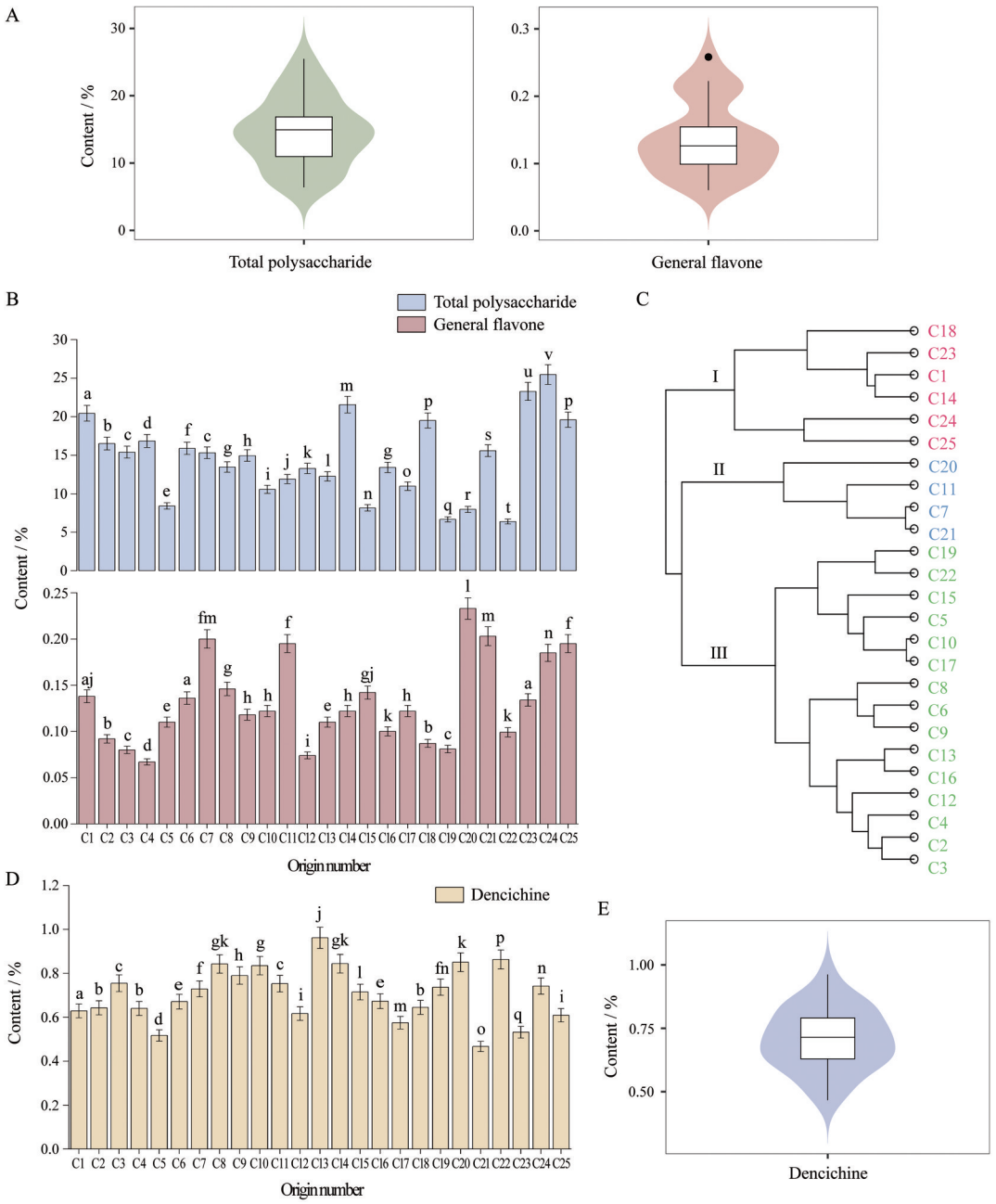


Figure 2　Analysis of total polysaccharides, general flavone, and dencichine content. A: Distribution of total polysaccharides and general flavone; B: Bar chart analysis of total polysaccharides and general flavone; C: Cluster analysis; D: Bar chart analysis of dencichine; E: Distribution of dencichine. $n = 25$, $\bar{x} \pm s$. Different letters indicate statistically significant differences at 0.05 level

参皂苷Re、三七皂苷R_4、人参皂苷Rf、人参皂苷Rb_1、人参皂苷Rc、人参皂苷Rh_1、人参皂苷Rd (图3A)。对25批三七样品指纹图谱与对照图谱进行相似度评价，其相似度范围在0.977～1.000 (图3B)，不同产地间三七样品整体皂苷类成分相似，药材质量较稳定。

不同产区三七皂苷含量存在差异 (图3C)，三七皂苷R_1含量范围0.516%～0.961%、人参皂苷Rg_1含量范围2.108%～3.621%、人参皂苷Re含量范围0.219%～0.566%、三七皂苷R_4含量范围0.062%～0.110%、人参皂苷Rf含量范围0.036%～0.102%、人参皂苷Rb_1含量

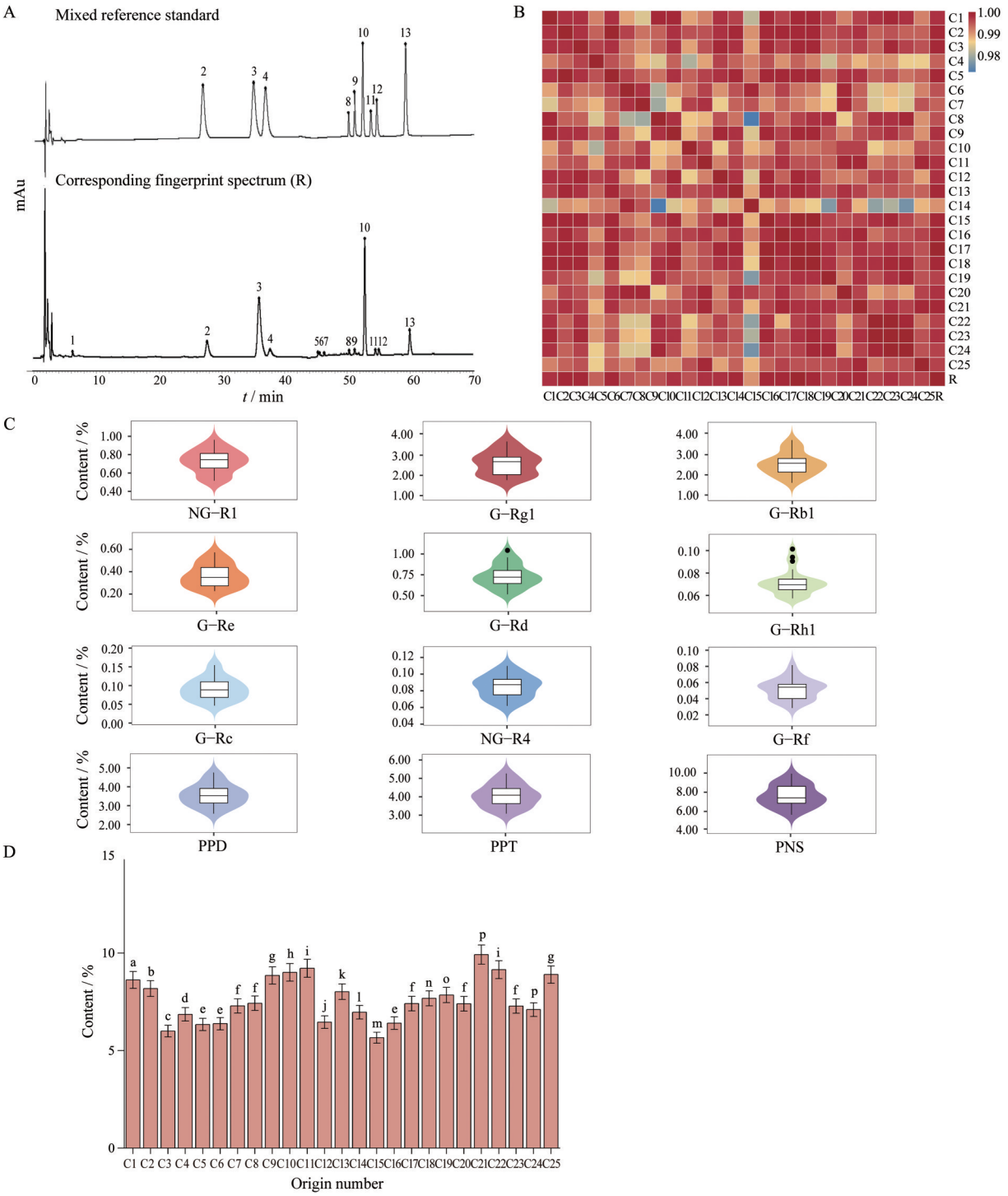


Figure 3 Saponins fingerprint, similarity, and content distribution analysis. A: Indicator component fingerprint spectrum; B: Similarity heatmap; C: Distribution of various saponins contents; D: Bar chart analysis of PNS. 2: NG-R1; 3: G-Rg1; 4: G-Re; 8: NG-R4; 9: G-Rf; 10: G-Rb1; 11: G-Rc; 12: G-Rh1; 13: G-Rd; 1, 5, 6, 7: Other common peaks. PNS: *Panax notoginseng* saponins; PPD: Panaxadiol saponin; PPT: Panaxatriol saponin. $n = 25$, $\bar{x} \pm s$. Different letters indicate statistically significant differences at 0.05 level

范围1.959%～3.723%、人参皂苷Rc含量范围0.046%～0.156%、人参皂苷Rh_1含量范围0.047%～0.102%、人参皂苷Rd含量范围0.507%～1.035%、人参二醇型皂苷含量范围2.578%～4.749%、人参三醇型皂苷含量范围3.083%～5.258%以及三七总皂苷含量范围5.661%～9.923%。其中, 来自云南文山州麻栗坡麻栗镇石岔村的三七中总皂苷含量较高 (图3D)。

4　不同产地三七皂苷类物质生物标志物的筛选

聚类热图结果表明不同产地的三七样品分为三大簇, 三七皂苷含量较高的产区主要分布在簇Ⅱ和Ⅲ中 (图4A)。为进一步分析不同产地三七的生物标志物, 以25批三七样品中9个皂苷含量为变量, 建立OPLS-DA模型 (图4B), 该模型$R^2Y = 0.776$, $Q^2 = 0.676$, 均大于0.5, 且$P < 0.005$, 表明该模型为有效模型 (图4C)。通过置换检验 (置换次数为200次), 随着置换保留度的下降, R^2和Q^2值下降, 且回归线呈向上的趋势, 说明置换检验过关, 该模型不存在过拟合现象, 可用于三七不同产地间皂苷物质的分析 (图4D)。结合变量重要性投影值 (variable importance for the projection, VIP), 以VIP > 1筛选出差异成分, 共筛选出5个差异标记物, 分别为人参皂苷Rc、人参皂苷Re、三七皂苷R_1、三七皂苷R_4和人参皂苷Rf, 为不同产地三七质量差异的主导皂苷成分 (图4E)。

5　不同产地三七多指标成分生物标志物的筛选

将淀粉、纤维素、木质素、黄酮、总糖、三七素和总皂苷含量为变量, 通过层次聚类, 25份三七样品分为三大簇 (图5A), OPLS-DA模型 (图5B) 的拟合参数$R^2Y = 0.773$, $Q^2 = 0.553$, 均大于0.5, 且$P < 0.005$, 表明该模型具有良好的拟合和预测能力 (图5C)。置换检验结果表明, 该模型不存在过拟合, 实验结果稳定可靠 (图5D)。结合VIP值, 以VIP > 1筛选出4个与三七产地相关的差异成分, 分别被鉴定为三七素、总多糖、支链淀粉和纤维素, 这些物质可以作为区分不同产区三七药材的潜在生物标志物 (图5E)。

6　三七药材成分与生态因子相关性分析

基于GMPGIS系统, 得到25个不同产地的19个生态因子, 涵盖了温度、湿度、降水等环境因素 (表3)。

19个生态因子与多种皂苷含量的相关性分析结

Table 3　Nineteen ecological factors from 25 production areas. AMT: Annual mean temperature; MDR: Mean diurnal range; ISO: Isothermality; TS: Temperature seasonality; MTWM: Max temperature of warmest month; MTCM: Min temperature of coldest month; TAR: Temperature annual range; MTWQ1: Mean temperature of wettest quarter; MTPQ: Mean temperature of driest quarter; MTWQ2: Mean temperature of warmest quarter; MTCQ: Mean temperature of coldest quarter; AP: Annual precipitation; PWM: Precipitation of wettest month; PDM: Precipitation of driest month; PS: Precipitation seasonality; PWQ1: Precipitation of wettest quarter; PDQ: Precipitation of driest quarter; PWQ2: Precipitation of warmest quarter; PCQ: Precipitation of coldest quarter

Origin number	AMT /℃	MDR /℃	ISO /℃	TS /℃	MTWM /℃	MTCM /℃	TAR /℃	MTWQ1 /℃	MTDQ /℃	MTWQ2 /℃	MTCQ /℃	AP /mm	PWM /mm	PDM /mm	PS /mm	PWQ1 /mm	PDQ /mm	PWQ2 /mm	PCQ /mm
C1	15.6	10.5	4.5	4.5	25.1	1.9	23.2	20.5	9.2	19.3	9.2	995.0	203.0	13.0	8.4	570.0	45.0	570.0	45.0
C2	14.0	10.4	4.7	4.1	22.8	0.9	21.9	18.6	8.2	19.1	8.2	1 076.0	232.0	14.0	8.8	631.0	43.0	631.0	43.0
C3	14.7	9.8	4.4	4.5	24.0	1.8	22.2	19.6	8.3	19.2	8.3	1 078.0	219.0	15.0	8.0	595.0	51.0	595.0	51.0
C4	15.2	10.4	4.6	4.2	24.3	2.1	22.2	19.9	9.2	19.4	9.2	1 038.0	219.0	14.0	8.5	600.0	45.0	600.0	45.0
C5	13.8	10.1	4.4	4.5	23.2	0.5	22.7	18.7	7.4	22.6	7.4	1 081.0	220.0	15.0	8.1	605.0	52.0	605.0	52.0
C6	13.6	9.5	4.0	5.0	23.1	(0.1)	23.2	19.2	6.5	18.6	6.5	1 174.0	223.0	15.0	8.3	652.0	49.0	652.0	49.0
C7	14.4	9.7	4.5	4.1	23.2	2.0	21.2	18.9	8.5	20.5	8.5	1 182.0	260.0	14.0	8.5	677.0	50.0	677.0	50.0
C8	16.3	9.9	4.4	4.5	25.6	3.5	22.1	21.0	9.8	20.0	9.8	1 014.0	206.0	13.0	7.9	556.0	48.0	556.0	48.0
C9	15.0	10.5	4.5	4.5	24.5	1.3	23.2	20.0	8.5	18.9	8.5	1 013.0	205.0	14.0	8.3	575.0	47.0	575.0	47.0
C10	16.3	8.6	3.5	5.6	27.1	3.2	23.9	22.6	8.4	20.2	8.4	1 352.0	260.0	18.0	7.8	724.0	66.0	724.0	66.0
C11	13.8	10.0	4.3	4.7	23.2	0.2	23.0	18.9	7.1	19.3	7.1	1 096.0	215.0	15.0	8.0	608.0	53.0	608.0	53.0
C12	14.3	10.3	4.4	4.6	23.6	0.4	23.2	19.4	7.8	19.1	7.8	996.0	203.0	12.0	8.4	570.0	42.0	570.0	42.0
C13	14.1	10.9	4.6	4.5	23.6	0.0	23.6	19.1	7.6	18.6	7.6	938.0	213.0	9.0	8.9	558.0	35.0	558.0	35.0
C14	14.1	9.5	4.5	4.2	23.0	1.9	21.1	18.6	8.1	19.9	8.1	1 207.0	265.0	14.0	8.5	689.0	51.0	689.0	51.0
C15	15.4	10.5	4.5	4.4	24.8	1.9	22.9	20.2	9.1	18.7	9.1	1 011.0	208.0	14.0	8.3	577.0	47.0	577.0	47.0
C16	13.8	10.2	4.3	4.9	23.4	(0.1)	23.5	19.3	6.9	20.8	6.9	979.0	193.0	11.0	8.4	553.0	38.0	553.0	38.0
C17	14.2	10.2	4.4	4.5	23.6	0.7	22.9	19.1	7.6	19.6	7.6	1 063.0	212.0	15.0	8.1	592.0	52.0	592.0	52.0
C18	14.3	10.3	4.4	4.6	23.8	0.8	23.0	19.3	7.8	21.0	7.8	1 058.0	209.0	15.0	8.0	588.0	52.0	588.0	52.0
C19	13.6	10.1	4.4	4.6	22.8	0.0	22.8	18.6	7.1	18.9	7.1	1 059.0	213.0	13.0	8.3	600.0	46.0	600.0	46.0
C20	16.1	10.4	4.6	4.3	25.4	3.2	22.2	20.8	10.1	18.6	10.1	1 013.0	212.0	13.0	8.4	582.0	45.0	582.0	45.0
C21	15.8	7.9	3.8	4.5	24.7	4.4	20.3	20.8	9.3	22.5	9.3	1 438.0	326.0	12.0	8.8	828.0	50.0	828.0	50.0
C22	17.4	8.1	3.9	4.6	26.6	6.0	20.6	22.4	10.8	22.4	10.8	1 352.0	303.0	11.0	8.6	772.0	49.0	772.0	49.0
C23	16.0	7.9	3.8	4.5	25.0	4.6	20.4	21.0	9.5	20.8	9.5	1 424.0	324.0	12.0	8.8	819.0	51.0	819.0	51.0
C24	17.4	8.2	3.9	4.6	26.7	6.0	20.7	22.5	10.9	21.0	10.9	1 342.0	299.0	11.0	8.6	765.0	49.0	765.0	49.0
C25	17.9	8.0	3.9	4.5	27.1	6.8	20.3	22.9	11.5	22.9	11.5	1 391.0	319.0	10.0	8.7	800.0	50.0	800.0	50.0

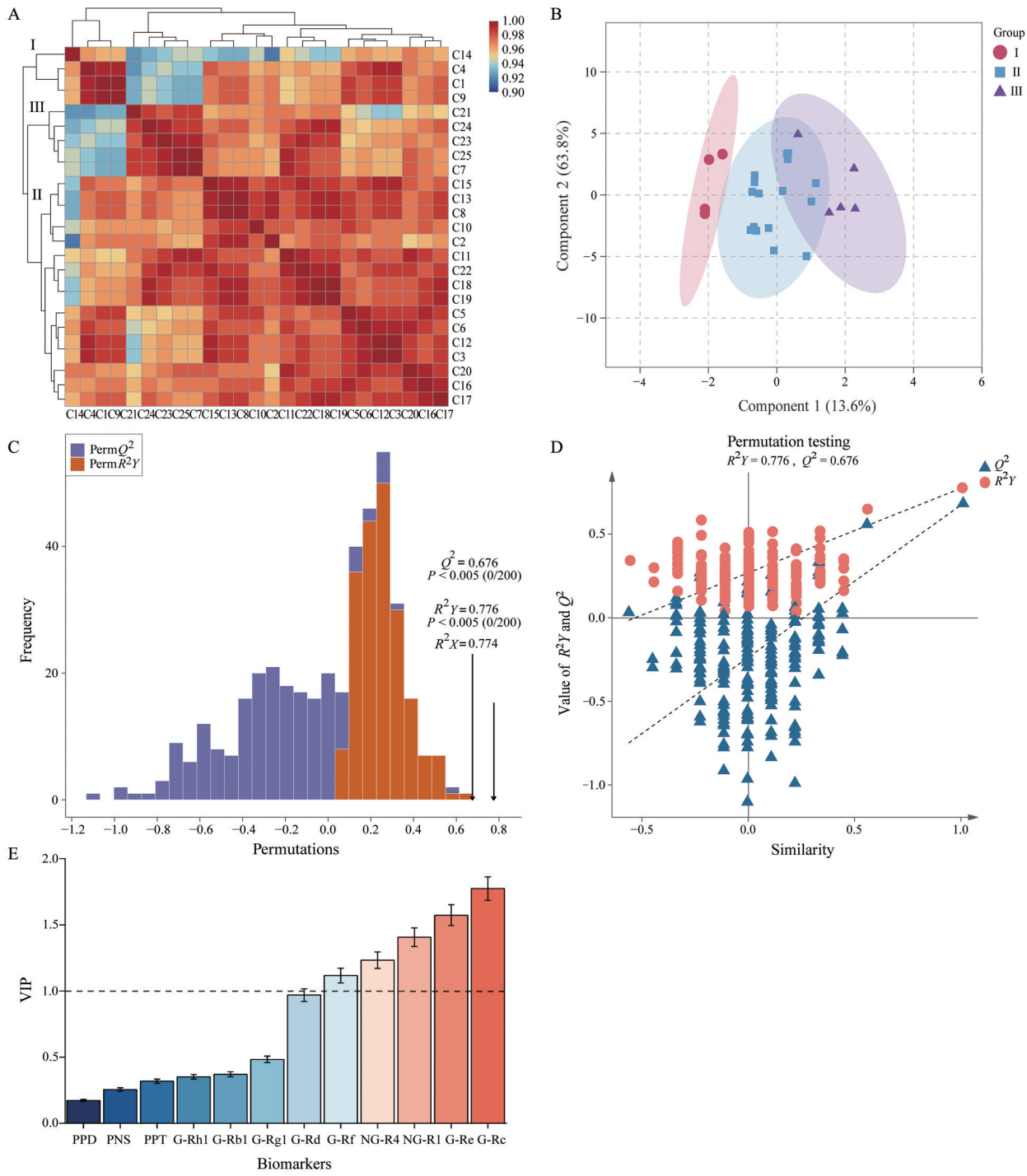


Figure 4 Comprehensive analysis of various saponins. A: Saponins clustering heatmap; B: Orthogonal partial least squares-discrimination analysis (OPLS-DA) of saponins; C: OPLS-DA model validation diagram of saponins; D: OPLS-DA displacement test chart of saponins; E: Variable importance for the projection (VIP) value. R^2X: Interpretation rate of the model for the X matrix; R^2Y: Interpretation rate of the model for the Y matrix; Q^2: The predictive power of the model; the closer these three prediction parameters are to 1, the more stable and reliable the model is

果表明, 人参皂苷Re、人参皂苷Rf、人参皂苷Rc以及人参皂苷Rd的含量积累与生态因子呈现显著正相关(图6A)。其中, 年降水量、等温性、平均月温差、最暖月份最高温度和最湿季度平均温度与人参皂苷Re的含量积累存在显著的正相关关系; 年降水量、平均月温差、最冷月份最低温度、气温年较差、最暖季度平均温度、最湿月份降水量、最湿季度降水量和最暖季度降水量与人参皂苷Rf的含量积累呈现显著正相关; 平均月

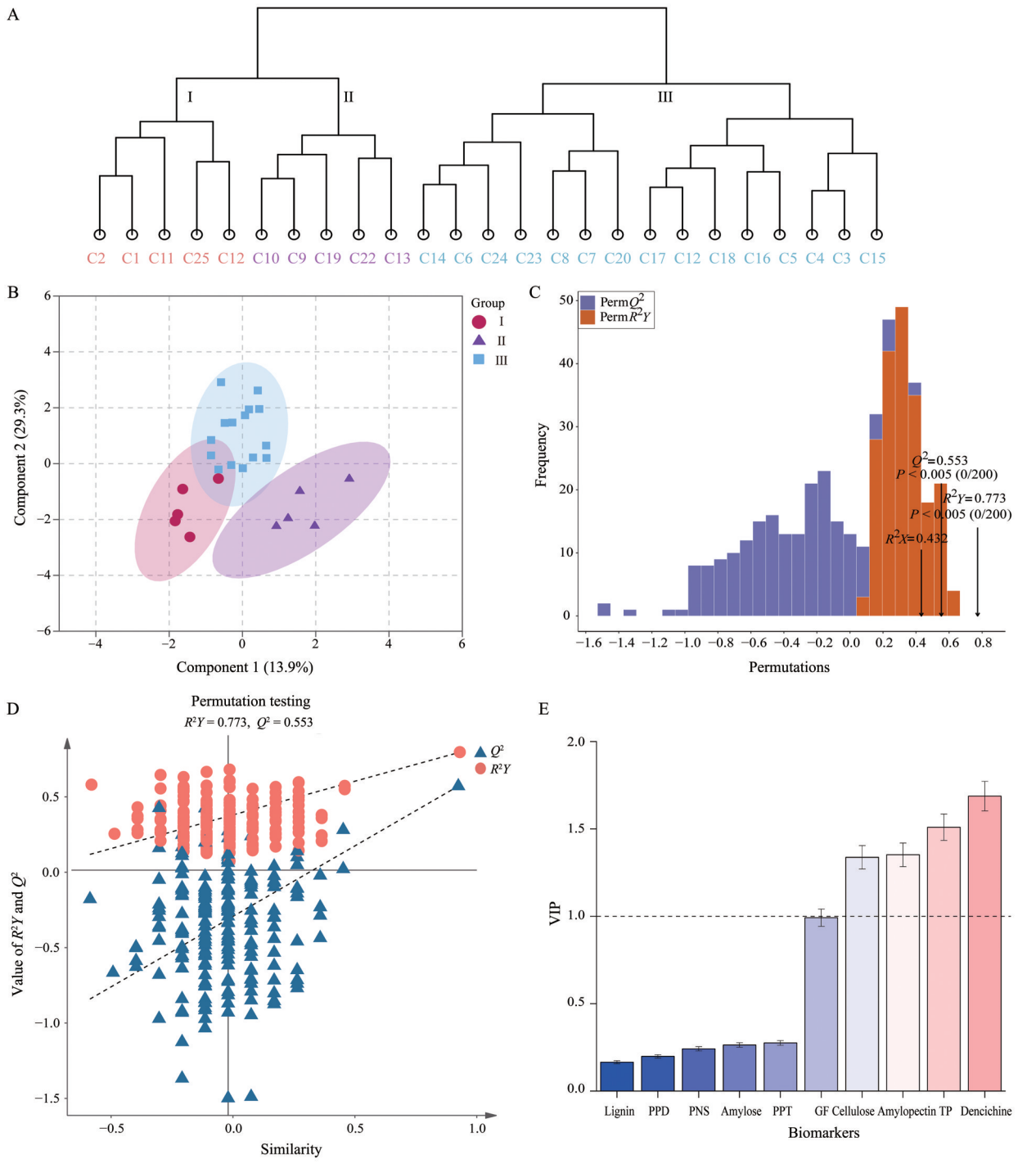


Figure 5　Multi component content analysis. A: Hierarchical clustering analysis; B: OPLS-DA of multi component; C: OPLS-DA model validation diagram of multi component; D: OPLS-DA displacement test chart of multi component; E: VIP value. TP: Total polysaccharide; GF: General flavone

温差、等温性、最冷月份最低温度、气温年较差、最暖季度平均温度、年降水量、最湿月份降水量、最湿季度降水量和最暖季度降水量与人参皂苷Rc的含量积累展现出显著的正相关趋势; 而人参皂苷Rd主要与等温性表现出显著的正相关关系 (图6B)。

生态因子与淀粉、木质素、纤维素、总多糖、总黄酮、三七素及皂苷类含量具有显著相关性。其中, 三七素含量与最湿季度降水量、最暖季度降水量、降水量季节性变化、年降水量、最暖季度平均温度和平均月温差等生态因子呈现显著性正相关 (图6C)。进一步可视化生态因子与各物质含量间的关系 (图6D), 结果表明, 降水量季节性变化和最干月份降水量显著影响了

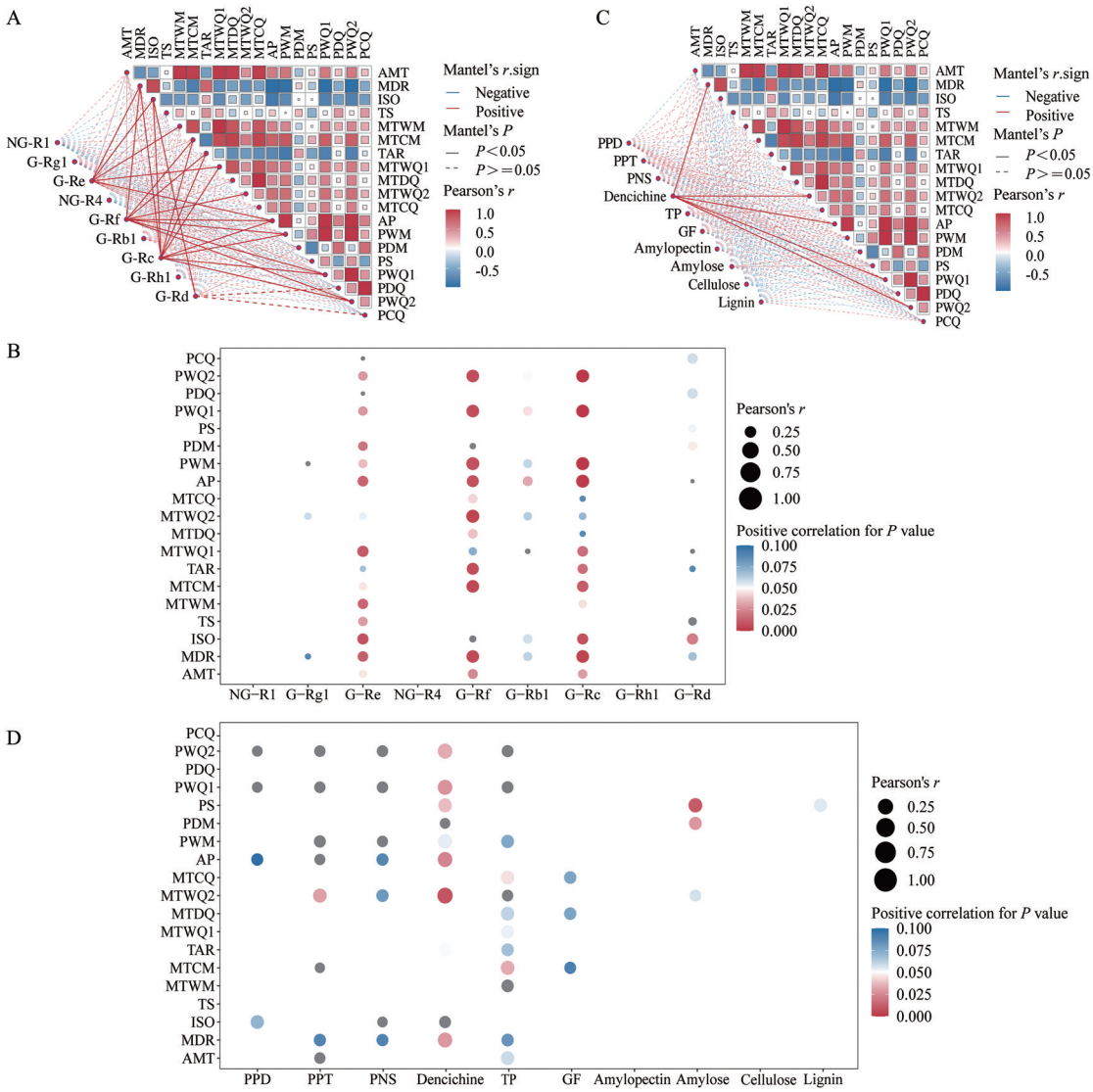


Figure 6 Correlation analysis between multiple components and ecological factors. A: Correlation analysis between saponins and ecological factors; B: Bubble chart of the correlation between saponins and ecological factors; C: Comprehensive analysis of multiple components and ecological factors; D: Bubble chart showing the correlation between multiple components and ecological factors

三七药材中直链淀粉含量; 最冷季度平均温度和最冷月份最低温度显著影响了三七药材中总多糖含量; 最暖季度平均温度显著影响了三七药材中原人参三醇型皂苷的含量积累。综上, 年降水量、平均月温差、等温性、最冷月份最低温度、最暖季度平均温度、最湿季度降水量以及最暖季度降水量, 显著影响了三七中多维度物质的含量, 对三七药材的物质积累过程发挥了重要作用, 这些生态因子可以作为影响三七药材质量的关键驱动因子。

讨论

三七作为传统名贵中药材, 药效成分复杂多样, 其主要活性成分为皂苷类物质。不同三七群体间三七皂苷存在差异, 这可能与产区、遗传背景、栽培管理等因素有关。Zhao 等[49]采用 RAPD 对 2 个不同产地的三七进行了 DNA 指纹图谱分析, 结果表明不同产地的三七具有不同遗传特征。Ying 等[50]利用“苗乡三七 1 号”“滇七 1 号”和自然群体为材料, 筛选出 41 对引物, 最终得到与表型、皂苷含量相关联的 8 对 SSR 标记引物, 结果表明三七遗传差异主要集中在居群内。Xian 等[51]概述了人参属药用植物种质资源, 确定不同地区三七居群间遗传分化水平程度较低, 遗传背景差异不显著的结果。同时, 研究发现不同土壤类型、不同产地的日照时数都影响皂苷含量[52]。本研究三七取样均为大田栽培模式, 不同产区三七皂苷、多糖、黄酮、三七素等存在差异, 结果表明产区是导致三七品质差异的关键因素

之一。

不同产区的三七中淀粉、木质素、纤维素、多糖、三七素和皂苷等含量具有地域性差异, 其中三七素、总多糖、支链淀粉和纤维素可以作为区分不同产区的潜在生物标志物。Feng 等[53]通过 HPLC-QAMS 多指标成分联合 OPLS-DA 及加权 TOPSIS 模型评价威灵仙药材质量的差异性, 得到齐墩果酸、威灵仙皂苷 C、灵仙新苷和常春藤皂苷元可能是影响威灵仙产品质量主要潜在标志物。Liu 等[54]研究表明基于多成分和模式识别分析, 可以筛选出不同产地金银花质量差异性的标志性成分, 为其产地鉴别及质量评价提供科学依据。Xiao 等[55]采用指纹图谱结合化学模式识别及多成分含量测定相结合, 对不同产地及不同居群鸡血藤药材进行综合评价, 获得了 6 个质量生物标志物。研究表明通过多指标成分分析结合化学模式识别等方法, 可以筛选出影响中药材质量的潜在生物标志物, 为评价药材道地性、优化种植选址及提升药材品质提供了科学依据。

药用植物的生长发育和有效成分积累受所处环境的影响[56], 其分布和生长取决于气候[57]、季节变化[58]和地理位置[59]等环境因素。然而, 环境因素之间也存在相互影响, 进而影响有效成分的积累。气候和土壤因素会驱动土壤微生物多样性和组成[60,61], 土壤微生物结构及多样性的差异影响了三七皂苷含量及地下生物量[62]。环境因素被认为是促进药用植物活性成分积累的核心原因之一。同一药用植物品种在不同产区的质量和产量差异显著[63]。气候因子对植株生长及有效成分的累积具有重要影响, 不同环境类型对中药基原植物有着复杂且密切的影响[64]。Yuan 等[65]对影响人字果药材内在质量的环境因子的相关性进行分析, 初步揭示影响人字果药材中次生代谢产物木兰花碱累积的主导环境因子。本研究结果表明生态因素影响三七药材中人参皂苷 Re、人参皂苷 Rf、人参皂苷 Rc、人参皂苷 Rd 和三七素含量的积累。其中, 年降水量、平均月温差、等温性、最冷月份最低温度、最暖季度平均温度、最湿季度降水量以及最暖季度降水量为驱动不同产区三七药材质量的关键生态因子。此外, 研究进一步探讨了环境因子与生物标志物如三七素、总多糖、支链淀粉、纤维素等含量间的关系, 三七素含量的积累主要受到降水量的影响, 总多糖含量的积累主要受到温度的影响。Zhang 等[66]研究发现, 人参皂苷的生物合成受环境调控, 通过实时荧光定量 PCR 检测显示, 皂苷含量、基因表达与生态因子相关, 光合有效辐射和土壤水势对根中皂苷积累影响较大。Liang 等[67]研究表明在干旱胁迫下党参根中海藻糖、棉子糖等多种碳水化合物显著积累, 通过上调可溶性淀粉合酶、纤维素合酶 A 催化亚基等生物合成途径中的纤维素酶 Korrigan 与关键基因表达, 表明这些碳水化合物可作为响应干旱胁迫的生物标志物。综上, 环境因子通过影响基因的表达进而影响有效成分的积累, 而支链淀粉、纤维素的含量积累基本与环境因子无关。

三七为历代本草著作和各版《中华人民共和国药典》所收载, 具有广泛的药理活性, 近年来对三七的研究取得了较大进展, 三七多方面的药用价值使其开发利用前景广阔[68]。Wang 等[69]基于“性状-化学成分”关联性分析, 整合非靶向定性分析与定量测定方法, 为三七品质评价提供新策略。Ding 等[70]采用 HPLC 法同时测定不同产地、不同商品规格三七样品中 5 种皂苷含量, 并结合灰色关联法建立整体质量评价模型。Song 等[71]研究中测定不同干燥条件下三七中三七素的含量, 揭示了三七素含量并不随干燥温度升高而线性变化的规律, 为制定三七中三七素的质量标准控制提供了重要依据。Ma 等[72]通过对三七不同蒸制时间的观察, 明确蒸制时间对三七总黄酮和多糖含量的影响。以往的研究多聚焦于皂苷、三七素、多糖及黄酮等传统药用有效成分的分析, 而对于三七中支链淀粉、直链淀粉、木质素及纤维素等大分子物质的研究和系统探讨则相对匮乏。本研究在沿袭对皂苷、三七素、多糖及黄酮等活性成分检测的基础上, 进一步探索了支链淀粉、直链淀粉、木质素及纤维素等大分子物质的定量分析, 系统地增加了三七成分分析维度。通过这一更为全面的质量评价框架, 科学地评估了三七药材的质量, 并强调了这些大分子物质在药材质量控制与资源开发中的独特价值与潜力, 为不同产地三七的质量评价及合理利用提供了坚实的科学依据。

综上, 本研究系统地分析了不同产区三七中的支链淀粉、直链淀粉、纤维素、木质素、总多糖、总黄酮、三七素以及多种皂苷成分。通过含量比较、指纹图谱构建和多元统计分析, 得到区分不同产区的潜在生物标志物。进一步应用 GMPGIS 系统获取了三七主产区 19 个生态因子, 评估了各生态因子对三七成分含量的影响, 并筛选出驱动不同产区三七药材质量的关键环境因子。本研究全面评价了不同产地三七质量, 并揭示了三七道地性及影响其形成的关键环境因子, 为后续三七质量控制和优化种植选址提供了有效参考和数据支撑。

在三七的后续研究中, 将聚焦于利用先进的分子生物学技术深入探究其品质形成及调控的分子机制, 同时构建涵盖多组分、多维度、高精度的质量评价体系, 以科技驱动提升三七的药用价值与市场竞争力, 促进三七产业的可持续发展。

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利益冲突: 所有作者均声明不存在利益冲突。

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