

清宫海桐皮本草考古: 古DNA信息与化学特征的共同证据

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摘要 海桐皮, 始载于五代《海药本草》, 药用历史悠久. 清朝御制医学著作《医宗金鉴》载有海桐皮汤. 仅依据文献史料难以考证古代海桐皮基原. 目前, 各地习作“海桐皮”的有4科6种, 亟待开展正本清源研究. 本文对故宫博物院藏清宫海桐皮药材文物(故233758)进行古DNA提取、建库, 运用鸟枪法宏基因组测序技术, 对清宫海桐皮古DNA进行分析, 将清宫海桐皮鉴定到芸香科花椒属*Zanthoxylum*植物. 利用UPLC-Q-TOF-MS/MS技术对清宫海桐皮化学特征进行分析, 共鉴定出20种化合物, 主要为生物碱类、黄酮类、木脂素以及香豆素类化合物, 结合现生样品比较, 与芸香科花椒属植物所含化学成分相似, 与古DNA分析结果互相佐证. 为进一步准确鉴定清宫海桐皮具体基原, 基于清宫海桐皮古DNA数据, 获取了长度为164 bp ITS、172 bp ETS和201 bp *trnH-psbA*序列, 分别构建清宫海桐皮与芸香科花椒属ETS、*trnH-psbA*、ITS与ETS联合序列系统发育树, 结果表明, 清宫海桐皮样品与椿叶花椒*Zanthoxylum ailanthoides* Sieb. et. Zucc.亲缘关系最近, 鉴定清宫海桐皮原植物为椿叶花椒*Z. ailanthoides*. 本文通过性状鉴定、分子考古与化学特征, 相互印证, 建立了清宫药材文物“科属-种”逐层鉴定基原的研究模式, 准确鉴定了清宫海桐皮基原, 为芸香科花椒属椿叶花椒*Z. ailanthoides*的树皮, 为实现海桐皮正本清源以及传承清宫医案提供了本草考古依据.

关键词 海桐皮, 本草考古, 分子考古, 化学特征

基原是药材的核心要素之一. 古往今来, 药材基原常发生变迁^[1]. 李时珍指出: “古今药物兴废不同”^[2]. 清代名医徐大椿在《医学源流论》^[3]发出感叹: “古今所用之药, 当时效验显著, 而本草载其功用凿凿者, 今依

方施用, 竟有应与不应, 其故何哉.” 药物基原的改变, 无疑对临床疗效产生重大影响. 马兜铃科植物关木通*Aristolochia manshuriensis* Kom.代替木通, 导致马兜铃酸中毒引起“关木通事件”^[4], 是药材基原变化对临床疗

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效影响的典型案例。因此,开展古代药材基原研究,可促进中医药的传承与发展,为中医临床疗效提供保障。

20世纪中叶以来,赵燏黄、谢宗万等中药学前辈将本草学研究与现代植物分类结合在一起,开创了本草考证的先河,对古代药物的基原开展了考证研究,取得了丰硕成果^[5,6]。但是本草考证主要依据文献史料,一些观点仅凭文献考证尚有争议。古代药物基原研究期待实物史料发现^[7]。2018年,作者提出本草学与考古学的交叉新领域——本草考古^[8]。本草文献考证与本草考古,分别依托间接史料与直接史料,相互补充,相互印证,为古代药物基原研究提供二重证据^[7]。

海桐皮,始载于五代李珣《海药本草》^[9]。宋《本草图经》记载:“海桐皮,出南海以南山谷,今雷州及近海州郡亦有之。叶如手大,作三花尖。皮若梓白皮而坚韧,可作绳,入水不烂。不拘时月采之”,并绘有“雷州海桐皮”图^[10]。有学者认为《本草图经》中“雷州海桐皮”为木棉科植物木棉*Bombax malabaricum* DC. (*Flora of China*收录为*Bombax ceiba* L.)^[11];也有学者认为《本草图经》所述“叶如手大,作三花尖”与五加科植物刺楸*Kalopanax septemlobus* (Thunb.) Koidz.相似^[12,13]。明代本草开始记载海桐皮的原植物树皮具钉刺,如《本草品汇精要》记载:“类梓白皮而有钉盖,擘之可脱”^[14]。《本草品汇精要》《本草纲目》《本草原始》等本草著作^[14-16]中绘有海桐皮原植物图,图中树干上均绘有明显的刺状物。明清时期,海桐皮广泛应用于中医临床。清朝御制医学著作《医宗金鉴》^[17]载有海桐皮汤,该方可专洗一切跌打损伤,筋翻骨错,疼痛不止。清宫医案有应用^[18,19]。目前,各地海桐皮的基原不尽相同,涉及4科6种,包括木棉科植物木棉*B. ceiba*^[11],五加科植物刺楸*K. septemlobus*^[12,13],豆科植物刺桐*Erythrina variegata* L.或乔木刺桐*E. arborescens* Roxb.^[20],芸香科植物椿叶花椒*Zanthoxylum ailanthoides* Sieb. et. Zucc.或花椒*Z. molle* Rehd.^[21]。它们的共同特征是树皮具刺。1977年版《中国药典》记载海桐皮来源于豆科刺桐*E. variegata* L. var. *orientalis* (L.) Merr. (*Flora of China*收录为*Erythrina variegata* L.)或乔木刺桐*E. arborescens* Roxb.的干燥树皮;川桐皮来源于五加科植物刺楸*K. septemlobus*的干燥树皮^[20]。但是,其后各版药典未再收载海桐皮。因此,探究古代海桐皮的基原,有助于传承经典名方和清宫医案,有助于提高临床疗效和用药安全。

宫廷作为中国封建社会最高统治者的居所和国家

权力中心之所在,清宫御药房用药考究,质量优良。故宫博物院珍藏有清宫御药房海桐皮药材文物,是研究清代海桐皮的重要实物史料。精准鉴定古代药材文物的基原是本草考古研究的重要任务之一。果实与种子类、叶类等植物遗存,由于具备相对丰富的形态学特征,可用于分类鉴定,成为植物考古分析中的常见研究对象^[22]。但是,皮类、根与根状茎类植物遗存,难以通过形态学特征及显微组织结构鉴定到准确的基原。

DNA作为遗传信息的载体,信息含量大,且不受外界环境因素和生物发育阶段及器官组织差异的影响,分子鉴定具有准确、用量少的独特优势。古DNA是指在古代生物遗骸(如化石、亚化石、博物馆收藏标本、考古学与法医学标本等)中的遗传物质。近年来,分子考古用于鉴定出土植物遗存,探索植物进化、生态和多样性起着重要作用^[23-27]。本文综合运用分子考古、化学特征,准确推断清宫御药房海桐皮的基原,为传承清宫医案提供本草考古依据。

1 材料和方法

1.1 材料

清宫御药房海桐皮药材文物(故233758)(图1)。收集了目前各地作“海桐皮”药用的4个科现生样品植物为对照(表1,图S1),包括芸香科植物椿叶花椒*Zanthoxylum ailanthoides* Sieb. et. Zucc.、花椒*Zanthoxylum molle* Rehd.、五加科植物刺楸*Kalopanax septemlobus* (Thunb.) Koidz.、木棉科植物木棉*Bombax ceiba* L.、豆科植物刺桐*Erythrina variegata* L.,均由中国中医科学院中药资源中心彭华胜教授鉴定。

1.2 清宫海桐皮药材文物DNA提取、建库及测序分析

(i) DNA提取、建库及测序。首先用超纯水清洗样品表面,去除表面的泥土灰尘,然后用10%的次氯酸钠溶液浸泡30 s,进一步除去其表面污垢和杂质,依次用超纯水、无水乙醇清洗、晾干。最后将样品进行液氮冷冻研磨成粉。DNA提取主要参考Japelaghi等人^[28]基于CTAB的方法,略有改进。测序文库构建参考Meyer等人^[29]的方法完成。DNA提取及建库过程中使用纯水作为空白对照来排除操作污染。上述实验均在中国中医科学院中药资源中心的本草考古分子实验室完成。测序文库通过Illumina HiSeq平台,使用PE150策略进行

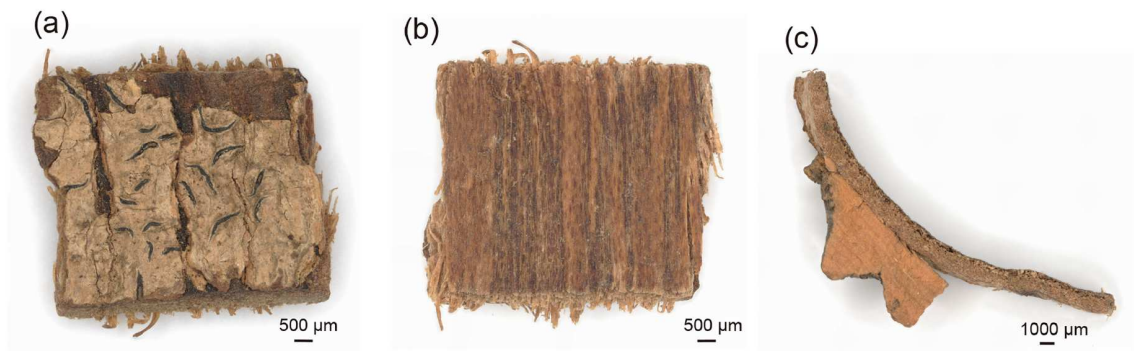


图 1 清宫海桐皮药材文物(故233758). (a) 外表面; (b) 内表面; (c) 断面
Figure 1 “Haitongpi” medicinal materials from imperial pharmacy in Qing Dynasty (GU233758). (a) External surface; (b) internal surface; (c) cross-section

表 1 现生样品信息

Table 1 Information on living specimens

现生样品	科名	样品产地
椿叶花椒 <i>Z. ailanthoides</i>	芸香科	广西壮族自治区桂宁市兴安县
椿叶花椒 <i>Z. ailanthoides</i>	芸香科	福建省宁德市福安市
朵花椒 <i>Z. molle</i>	芸香科	安徽省清凉峰国家级自然保护区
朵花椒 <i>Z. molle</i>	芸香科	安徽省黄山市祁门县历口镇
刺楸 <i>K. septemlobus</i>	五加科	安徽省合肥市磨店乡
木棉 <i>B. ceiba</i>	木棉科	广东省广州市
刺桐 <i>E. variegata</i>	豆科	福建省泉州市

鸟枪法测序(shotgun sequencing).

(ii) 数据分析. 首先对测序下机序列进行质量控制^[30]. 使用AdaptorRemoval (v2.3.2)^[31]去掉接头和Ns, 合并双端数据并移除低质量序列. 通过fastp (v0.23.2) 剪切序列末端poly-X, 去除重复及低复杂度序列^[32]. 最后运行sga^[33]进一步过滤掉低复杂度和长度小于30的序列, 并通过vsearch^[34]进一步去除重复序列. 同时构建本地数据库, 包括NCBI (<https://www.ncbi.nlm.nih.gov/>) 的全核酸数据库NCBI-nt (2023年7月31日版本)和NCBI-RefSeq (release 218). 将质控后的数据通过bowtie 2与本地数据库进行比对分析, 并通过samtools程序^[35]将生成的sam文件转换为bam文件并排序. 对于存在多次比对的序列, 使用ngsLCA (v1.0.5)^[36]计算最近公共祖先, 设置参数使每次比对允许的原始序列和参考基因组的最大错配数为2. 根据获得的植物物种注释结果, 对样品进行科属鉴定. 之后, 将注释得到的植物基因序列通过NCBI blast与参考物种的基因序列进行再次比对, 获得清宫海桐皮药材文物DNA条形码片段, ITS、ETS和*trnH-psbA*序列.

1.3 清宫药材文物样品与现生样品UPLC-Q-TOF-MS/MS分析

(i) 仪器与材料. Acquity UPLC-I-Class 超高效液相串联Synapt-XS IMS Q-TOF高分辨质谱仪, 配有Mas-slynx 4.2质谱工作站(Waters, 美国)、GL2241-ISCN分析天平(Sartorius, 德国)、SB-800-DTD超声波清洗仪(宁波新芝生物科技股份有限公司)、离心机(Eppendorf, 德国)、JXFSTPRP-48L全自动样品快速球磨仪(上海净信实业发展有限公司)、移液枪(Eppendorf, 德国)、1.5 mL离心管(Axygen, 美国)和4 mL透明螺口玻璃瓶(北京赛谱锐思科技有限公司).

(ii) 试剂: 甲酸、乙腈、甲醇(色谱纯, Merck, 德国), 超纯水(屈臣氏). 对照品: 橙皮苷Hesperidin (CAS No.: 520-26-3, 批号: 21120608)、美洲花椒素Xanthyletin (CAS No.: 553-19-5, 批号: 24061154)均购自北京倍特仁康生物医药科技有限公司, 芝麻素Sesamin (CAS No.: 607-80-7, 批号: C07M7H10462)、木兰花碱Magnoflorine (CAS No.: 2141-9-5, 批号: A18I13223349)均

购自上海源叶科技有限公司, 所有对照品纯度均>98%.

(iii) 样品处理. 分别取少量清宫海桐皮样品及现生样品, 将粉碎后样品粉末精密称定并转移至4 mL储样瓶中, 加入2 mL色谱甲醇, 超声提取30 min, 12000 r/min离心10 min, 取上清液用色谱甲醇稀释至5.0 mg/mL, 即得供试品溶液, 备用. 另取500 μ L色谱甲醇加入1.5 mL无菌离心管中, 同等条件处理, 即得空白对照溶液. 分别取橙皮苷、美洲花椒素、芝麻素和木兰花碱对照品适量, 精密称定, 加色谱甲醇溶解, 分别制得浓度为1 mg/mL单一对照品储备液, 混合对照品储备液为0.2 mg/mL, 均置于4 $^{\circ}$ C保存.

(iv) 色谱条件. 色谱柱为Acquity PRM BEH C18 (2.1 mm $\times$ 100 mm, 1.7 μ m); 流动相A: 0.1%甲酸-水溶液; 流动相B: 0.1%甲酸-乙腈溶液; 梯度洗脱(0~1 min, 90% A; 1~12 min, 90%~75% A; 12~22 min, 75%~45% A; 22~24 min, 45%~40% A; 24~26 min, 40%~20% A; 26~27 min, 20% A; 27~28 min, 20%~90% A; 28~30 min, 90% A); 柱温40 $^{\circ}$ C; 流速0.3 mL/min, 进样量1 μ L.

(v) 质谱条件. 数据采集模式: ESI正、负离子模式, MS^E continuum采集模式; 毛细管电压2.5 kV (-) /0.5 kV (+); 锥孔电压 40 V, 除溶剂气体为氮气, 流速 900 L/h, 除溶剂温度 450 $^{\circ}$ C, 离子源温度100 $^{\circ}$ C; 扫描范围 m/z 50~1500 Da, 碰撞气体为氩气; 正离子模式下高能量扫描碰撞能量为20~40 eV, 负离子模式下高能量扫描碰撞能量为40~60 eV; 准确质量数用亮氨酸脑啡肽做校正液(负离子554.2615, 正离子556.2771).

1.4 现生样品DNA提取、PCR扩增及测序

(i) DNA提取. 各取收集的椿叶花椒与朵花椒干燥样品叶片约30 mg, 置于装有磁珠的无菌离心管中, 液氮速冻研磨. 采用QIAGEN植物基因组DNA小提试剂盒DNeasy Plant Mini Kit提取现生样品总DNA. 经酶标仪检测浓度和纯度, 使用1%琼脂糖凝胶电泳法检测提

取DNA的质量, 确保DNA样本满足PCR扩增需求. 提取的DNA样品置于-20 $^{\circ}$ C冰箱中保存.

(ii) PCR扩增及测序. 选取ITS、ETS及\muL: 2 $\times$ M5 Super FastTaq PCR MasterMix 10 μ L, 引物F和引物R (10 μ mol/L)各0.5 μ L, DNA模板2 μ L, ddH₂O 7 μ L. PCR扩增程序: 95 $^{\circ}$ C预变性3 min, 94 $^{\circ}$ C 10 s, 56 $^{\circ}$ C 15 s, 72 $^{\circ}$ C 10 s, 30个循环, 72 $^{\circ}$ C延伸5 min. PCR产物经1%琼脂糖凝胶电泳检测. PCR产物交由生工生物工程(上海)股份有限公司进行回收、纯化及测序.

1.5 系统发育分析

基于本研究得到的DNA序列与GenBank中下载的芸香科植物ITS、ETS、*trnH-psbA*序列数据(表S1), 分别以芸香科植物吴茱萸*Tetradium ruticarpum* (A. Juss.) T. G. Hartley、宜昌橙*Citrus cavaleriei* H. Lév. ex Cavalier、*C. limetta* Risso作为外类群^[42,43], 使用PAUP*4.0b10^[44]、raxmlGUI2.0^[45]、MrBayes 3.2.7^[46]分别对ETS、*trnH-psbA*、ITS与ETS联合序列构建最大简约树(MP树)、最大似然树(ML树)和贝叶斯推断树(BI树). 用FigTree v1.4.4^[47]软件对已构建的系统发育树进行可视化.

2 结果

2.1 清宫海桐皮药材文物的性状特征

清宫海桐皮药材文物样品(故233758)呈薄板状或长条状, 略卷曲. 外表面黑灰色或淡黑灰色, 并有灰白色斑纹, 多皱缩纵条纹及纵向凹纹(图1(a)). 内表面黄白色或黄棕色, 光滑, 有细纵纹(图1(b)). 有的具乳头状钉刺, 刺横切面断面呈现(图1(c)). 横切面不光滑, 呈现纤维状. 可判断该植物样品为某种木本植物的树皮.

表 2 ITS、ETS和

Table 2 Primers of ITS2, ETS and *trnH-psbA*

引物名称	正反引物	引物序列(5'→3')	参考文献
ITS	ITS1F	TCCGTAGGTGAACCTGCGG	[37]
	ITS4R	TCCTCCGCTTATTGATATGC	
ETS	F	ATAGAGCGCGTGAGTGGTG	[38]
	R	GACAAGCATATGACTACTGGCAGGATCAA	[39]
<i>trnH-psbA</i>	F	CGCGCATGGTGGATTCAATCC	[40]
	R	GTTATGCATGAACGTAATGCTC	[41]

2.2 清宫海桐皮药材文物的科属鉴定

2.2.1 分子考古

清宫海桐皮药材文物样品古DNA测序共获得56785428条reads, 经过质量控制共获得50033142条有效reads, 其中788035条序列可进行物种注释. 去除空白样品中存在的污染序列(空白中检测到的物种序列), 及微生物的物种信息, 清宫海桐皮样品古DNA分析主要注释到芸香科花椒属*Zanthoxylum*植物.

2.2.2 化学特征

对清宫海桐皮药材文物样品进行化学特征分析, 根据化合物质谱裂解规律及标准品对比分析共鉴定出20个化合物(图2和表3), 包括14个生物碱类、4个黄酮及其苷类、1个木脂素类及1个香豆素类(图3). 由此可知, 清宫海桐皮药材文物样品中主要特征成分为生物碱类化合物, 按照生物碱结构类型可分为苯异喹啉类

(化合物14, 16~17, 19~20), 苯环酰胺类(化合物1和12), 阿朴啡类(化合物2和4), 喹啉类(化合物9和10), 小檗碱类(化合物7和8)以及2-喹啉酮类(化合物3), 为芸香科花椒属植物中的主要特征性化合物^[48]. 为验证清宫海桐皮药材文物样品是否为芸香科花椒属植物, 以芸香科花椒*Z. molle*、五加科刺楸*K. septemlobus*、木棉科木棉*B. ceiba*及豆科刺桐*E. variegata*的树皮为对照样品, 在正离子模式下分别采集质谱数据(图2). 结果表明, 清宫海桐皮特征性化合物与现生样品芸香科植物花椒的树皮相似(图2), 推断清宫海桐皮为芸香科花椒属植物, 与分子考古结果相互印证.

2.3 清宫海桐皮药材文物的种质鉴定

基于性状特征、分子考古及化学特征, 推断清宫海桐皮为芸香科花椒属植物的树皮. ITS、ETS、*trnH*-

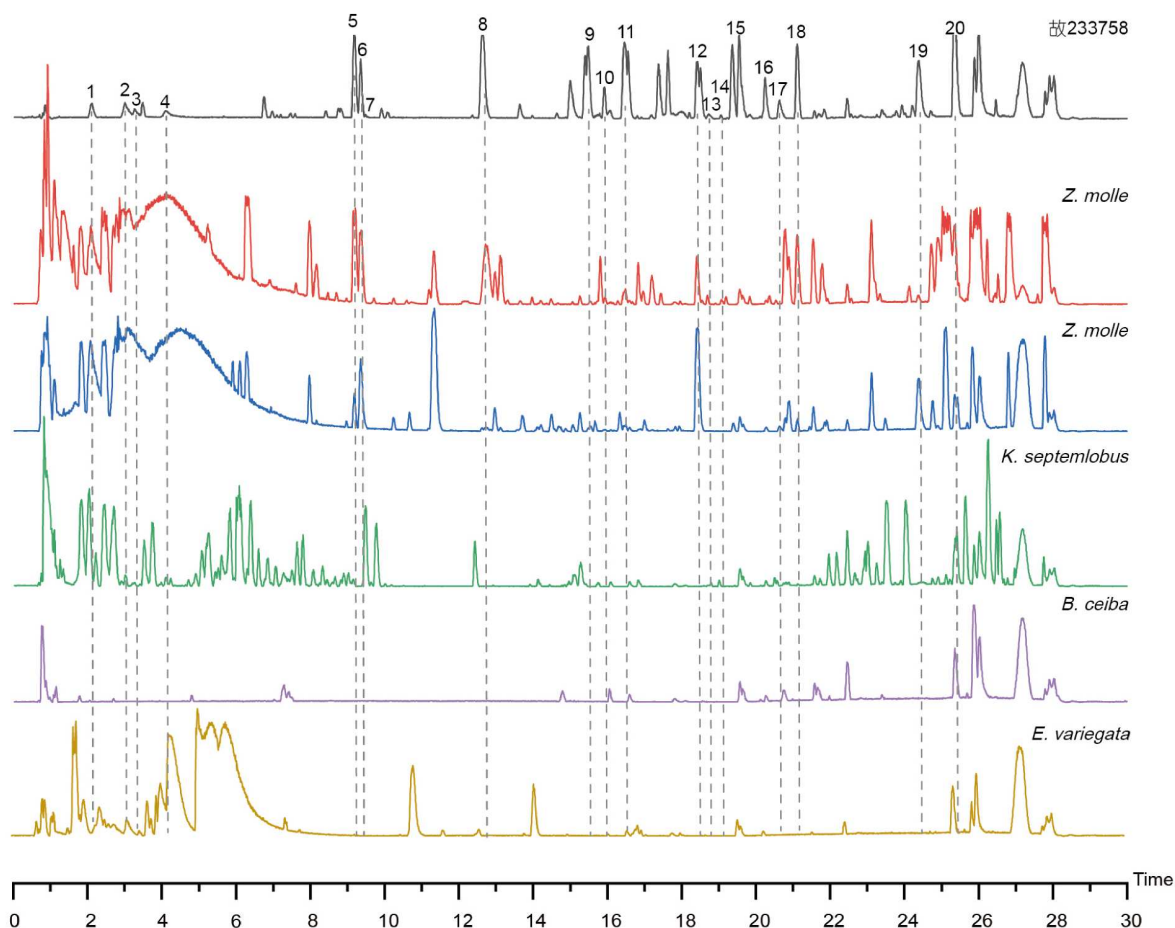


图2 清宫海桐皮(故233758)与现生样品UPLC-Q-TOF-MS/MS在正离子模式下总离子流图

Figure 2 The basic peak ion chromatogram of "Haitongpi" from imperial pharmacy in Qing Dynasty (GU233758) and modern samples by UPLC-Q-TOF-MS/MS in positive ion mode

表 3 基于UPLC-Q-TOF-MS/MS鉴定清官海桐皮(故233758)中的化学成分

Table 3 Identification of chemical components in “*Haitongpi*” from Qing Dynasty Palace (GU233758) using UPLC-Q-TOF-MS/MS

No	保留时间 (min)	实测值 <i>m/z</i>	分子式	加和离子	误差 (ppm)	MS/MS碎片离子	化合物鉴定 ^{a)}	结构类型	植物来源	文献
1	2.14	356.1521	C ₂₀ H ₂₂ NO ₅	[M+H] ⁺	6.5	344.1869, 314.1788, 298.1455	Zanthosin	生物碱类	<i>Zanthoxylum nitidum</i>	[49]
2	3.01	342.1724	C ₂₀ H ₂₄ NO ₄ ⁺	[M] ⁺	5.6	297.1134, 282.0887, 265.0878, 237.0884, 222.0695, 219.0799, 209.0963, 207.0809, 205.0652, 194.0742, 191.0854, 179.0890, 177.0789, 165.0717, 153.0677	Magnoflorine A	生物碱类	<i>Z. nitidum</i>	[50~52]
3	3.26	328.1920	C ₂₀ H ₂₆ NO ₃	[M+H] ⁺	2.1	297.1134, 283.1358, 265.0878, 189.0913, 107.0482	Simulansine	生物碱类	<i>Z. simulans</i>	[53]
4	4.13	342.1724	C ₂₀ H ₂₄ NO ₄ ⁺	[M] ⁺	5.6	297.1134, 282.0887, 265.0878, 237.0938, 222.0695, 219.0799, 209.0963, 207.0759, 205.0652, 194.0742, 191.0854, 179.0842, 177.0696, 165.0717, 153.0721	Magnoflorine B [*]	生物碱类	<i>Z. nitidum</i>	[50,51]
5	9.18	609.1835	C ₂₈ H ₃₃ O ₁₅	[M+H] ⁺	2.5	463.1257, 301.0755, 287.0516, 286.0449, 153.0546, 149.0625	Diosmin	黄酮苷类	<i>Z. nitidum</i>	[50,54]
6	9.38	611.2003	C ₂₈ H ₃₅ O ₁₅	[M+H] ⁺	4.4	465.1349, 303.0878, 286.0687, 285.0755, 153.0198, 149.0582	Hesperidin [*]	黄酮苷类	<i>Z. nitidum</i>	[50]
7	9.53	354.1691	C ₂₁ H ₂₄ NO ₄ ⁺	[M] ⁺	-4.0	190.0895	<i>N</i> -methylcanadine isomer	生物碱类	<i>Z. veneficum</i>	[55]
8	12.63	354.1691	C ₂₁ H ₂₄ NO ₄ ⁺	[M] ⁺	-4.0	190.0895	<i>N</i> -methylcanadine	生物碱类	<i>Z. veneficum</i> ; <i>Z. brachyacanthum</i>	[55]
9	15.47	230.0804	C ₁₃ H ₁₂ NO ₃	[M+H] ⁺	-5.7	215.0616, 200.0368, 186.0549, 172.0389, 158.0626, 144.0441, 116.0485	γ -Fagarine	生物碱类	<i>Z. nitidum</i>	[50]
10	15.00	299.0569	C ₁₆ H ₁₁ O ₆	[M-H] ⁻	4.3	299.0569, 284.0336	Kaempferide	黄酮类	<i>Z. bungeanum</i> ; <i>Z. schinifolium</i>	[56]
11	16.46	200.0716	C ₁₂ H ₁₀ NO ₂	[M+H] ⁺	2.0	185.0530, 156.0427, 129.0601, 102.0469	Dictamnine	生物碱类	<i>Z. nitidum</i>	[50]
12	18.42	292.1300	C ₁₇ H ₁₉ NO ₂ 2Na	[M+Na] ⁺	-4.5	270.1540, 135.0817	<i>N</i> -(4-methoxyphenethyl)- <i>N</i> -methylbenzamide	生物碱类	<i>Z. ailanthoides</i>	[57]
13	18.69	403.1409	C ₂₁ H ₂₃ O ₈	[M+H] ⁺	4.0	388.1130, 373.0918, 348.1219, 301.0694, 183.0287	(7' <i>S</i> , 8' <i>S</i>)-Bilagrewin	黄酮类	<i>Z. avicennae</i>	[58]
14	19.06	382.1323	C ₂₁ H ₂₀ NO ₆	[M+H] ⁺	8.4	364.1194, 354.1360, 339.1132, 292.0699, 277.0518, 264.0689, 201.0784, 167.0353	Isoarnottianamide/ Arnottianamide	生物碱类	<i>Z. nitidum</i>	[49,59]
15	19.36	229.0886	C ₁₄ H ₁₃ O ₃	[M+H] ⁺	8.7	213.0587, 199.0376, 185.0577, 171.0434, 169.0654, 159.0774, 157.0643, 155.0858, 143.0490, 131.0507, 115.0536, 103.0554, 91.0550	Xanthyletin [*]	香豆素类	<i>Z. avicennae</i>	[60~62]
16	20.20	364.1194	C ₂₁ H ₁₈ NO ₅	[M+H] ⁺	2.5	349.0944, 348.1219, 334.0707, 320.0948, 306.0789, 290.0765	Oxynitidine	生物碱类	<i>Z. nitidum</i>	[50]
17	20.65	320.0948	C ₁₉ H ₁₄ NO ₄	[M+H] ⁺	7.8	305.0700, 277.0753, 248.0719, 219.0695, 201.0435	Zanthoxyline	生物碱类	<i>Z. nitidum</i>	[59]
18	21.12	377.0995	C ₂₀ H ₁₈ O ₆ Na	[M+Na] ⁺	1.6	355.1168, 353.1036, 337.1071, 323.0925, 319.0945, 289.0884, 261.0922, 231.0796, 203.0845, 135.044977.0393	Sesamin [*]	木脂素类	<i>Z. armatum</i>	[63~65]
19	24.37	350.1406	C ₂₁ H ₂₀ NO ₄	[M+H] ⁺	4.0	334.1093, 319.0819, 304.0934, 290.0825, 247.0751, 219.0799	Dihydronitidine	生物碱类	<i>Z. nitidum</i>	[50]
20	25.34	350.1406	C ₂₁ H ₂₀ NO ₄	[M+H] ⁺	4.0	334.1093, 319.0819, 304.0934, 290.0825, 247.0751, 219.0799	Dihydrochelerythrine	生物碱类	<i>Z. nitidum</i>	[50]

a) *, 与对照品比对的化合物(图S2~S7)

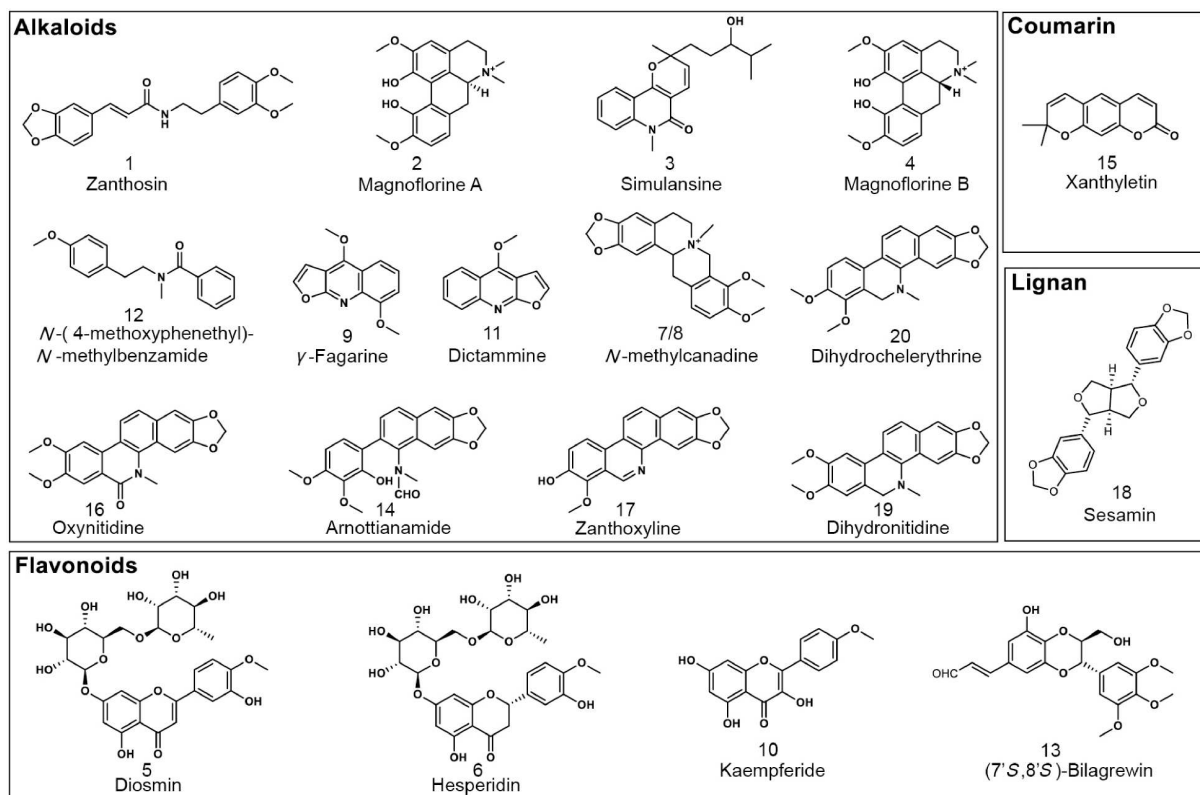


图3 清宫海桐皮(故233758)中化合物结构图

Figure 3 Compound structure diagram in “Haitongpi” from imperial pharmacy in Qing Dynasty (GU233758)

psbA, ETS与ITS联合序列在花椒属物种水平可以有效实现鉴别^[66,67]。基于清宫海桐皮样品古DNA数据, 获取了长度为164 bp ITS、172 bp ETS和201 bp *trnH-psbA*。

从GenBank下载7种崖椒亚属(Subgen. *Fagara*)和4种花椒亚属(Subgen. *Zanthoxylum*)的ETS、ITS、*trnH-psbA*序列, 涵盖了目前已公布的花椒属2个亚属相关的参考数据(表S1)。基于ETS、*trnH-psbA*的系统发育树, 结果表明清宫海桐皮(故233758)与椿叶花椒 *Z. ailanthoides* 和朵花椒 *Z. molle* 亲缘关系较近(图S8和S9)。用ITS与ETS联合序列构建系统发育树, 结果表明清宫海桐皮(故233758)与椿叶花椒 *Z. ailanthoides* 亲缘关系最近(图4)。三种常用的系统发育推断方法(MP、ML、BI)分别构建的ITS与ETS联合序列系统树在拓扑结构基本一致, 花椒属两个亚属分别聚类为两个独立的类群, 并且现生花椒属相同种表现为单系(图4), 表明系统发育树结果可靠。通过对清宫海桐皮的ITS、ETS及 *trnH-psbA* 序列与椿叶花椒序列比对分析一致(图5)。

综上, 可以鉴定清宫海桐皮来源于芸香科植物椿叶花椒 *Z. ailanthoides* 的树皮。

3 讨论

3.1 多种方法相互印证, 建立未知药材文物“科属-种”逐层鉴定基原的研究模式

故宫博物院藏清宫御药房药物文物种类丰富。有的可以通过性状就可以鉴定到种, 如茯苓^[68,69]、三七、人参。有的药材, 尤其是根与根状茎类、皮类、茎木类药材, 常常不同科属植物具有相似的性状特征, 难以通过性状鉴定科属。

药材的基原鉴定是指应用植(动、矿)物的分类学知识, 对中药的来源进行鉴定研究, 确定其正确的学名, 以保证应用品种准确无误, 又称来源鉴定^[70]。基原, 常包括中药的科名、植(动、矿)物名称、拉丁学名和药用部位。药材的基原鉴定是中药鉴定工作的基础, 也是中药生产、临床用药的依据。对于性状鉴定可以判断科或属的药材文物, 可以针对这个科属的分子鉴别特征, 设计相关引物, 从而快速实现基原鉴定^[71]。

对于性状难以鉴定科属的药材, 科属判别非常重要。如果科属判别错误, 就会差之毫厘, 谬之千里, 导

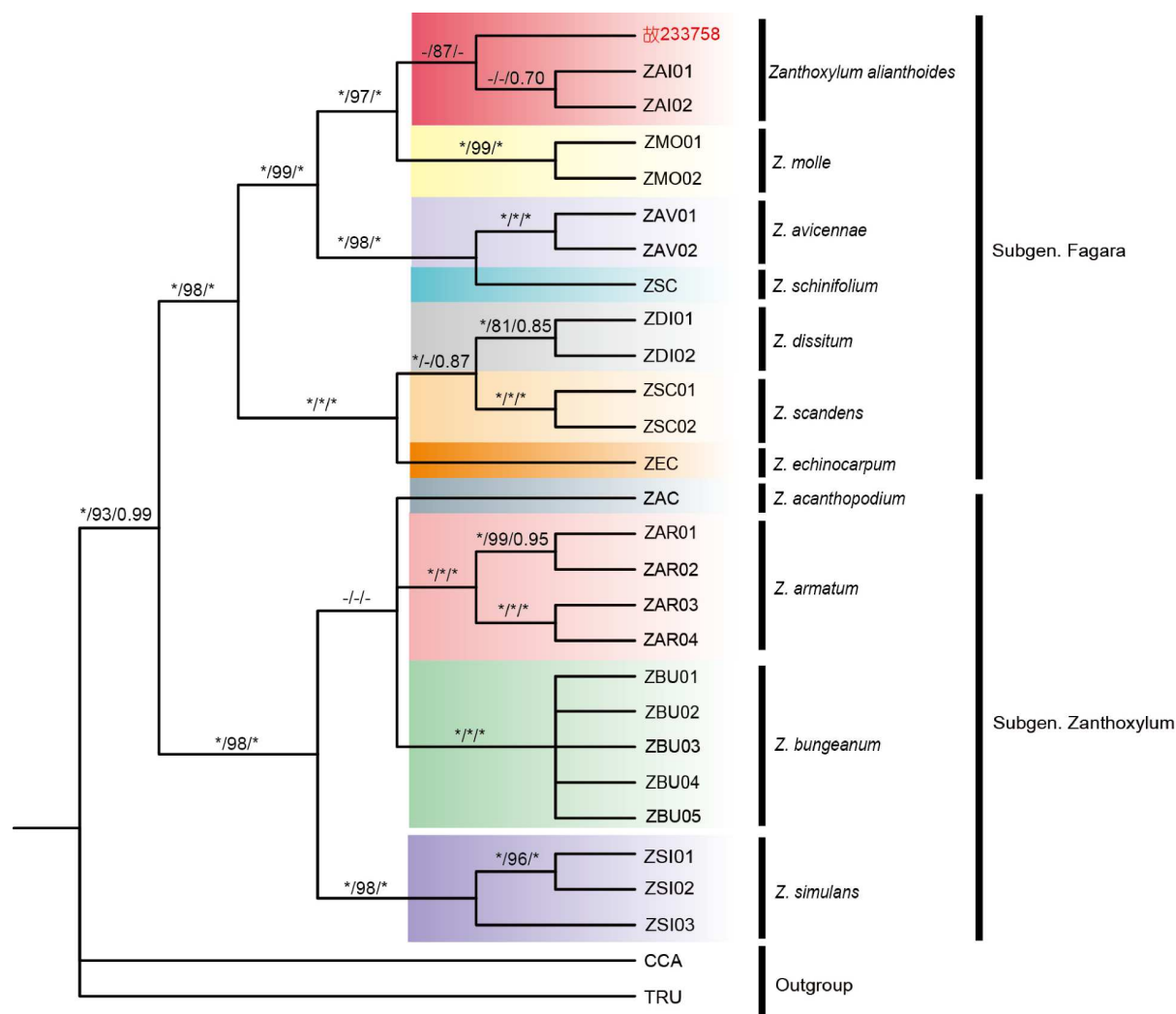


图 4 基于ETS与ITS联合序列构建的清宫海桐皮(故233758)与花椒属植物的系统发育树。分支上的数字分别代表MP BS、ML BS和BI PP, 枝上的“-”表示BS<70%, “*”表示BS为100%或PP为1.00。

Figure 4 Phylogenetic reconstruction of “*Haitongpi*” from imperial pharmacy in Qing Dynasty (GU233758) and *Zanthoxylum* based on the nuclear markers ETS and ITS. The numbers on the branches represent MP BS, ML BS, and BI PP, respectively. The “-” on the branches indicates that BS is less than 70%, and “*” indicates that BS is 100% or PP is 1.00

致基原准确鉴定失败。鸟枪法宏基因组技术可以高效地识别样品中的生物遗传物质, 从而分析样品的生物群落组成。近年来, 鸟枪法宏基因组技术在重构沉积物古DNA动植物群落中得到广泛应用^[72,73]。该技术的应用需要借助建立相应的核酸数据库, 所建立的核酸数据库是否可以完全覆盖到鉴别对象, 这会影响物种鉴定的准确性。但是应用该方法, 有助于推断出清宫海桐皮药材文物为芸香科花椒属植物, 为后续进一步鉴定提供参考依据。

药材文物中除了含有古DNA信息外, 其所含的化学成分也有助于推断科属。尽管药材文物中所含的化

学成分因时间变化可能发生降解或减少, 但是其所含的有机残留物可以为推断相应科属提供证据。Ren等人^[74]通过有机残留物推断法门寺出土唐代香料中有沉香和乳香。本研究利用UPLC-Q-TOF-MS/MS分析技术从清宫海桐皮中鉴定出20种化合物, 其中主要是生物碱类、黄酮类、木脂素以及香豆素类化合物, 结合文献与现生样品对照确定其与芸香科花椒属植物所含化学成分相似。综合清宫海桐皮的分子特征和化学特征, 相互印证, 共同推断其来源于芸香科花椒属植物的树皮, 为样品的科属推断提供了坚实的证据。

对花椒属植物而言, ITS、ETS和

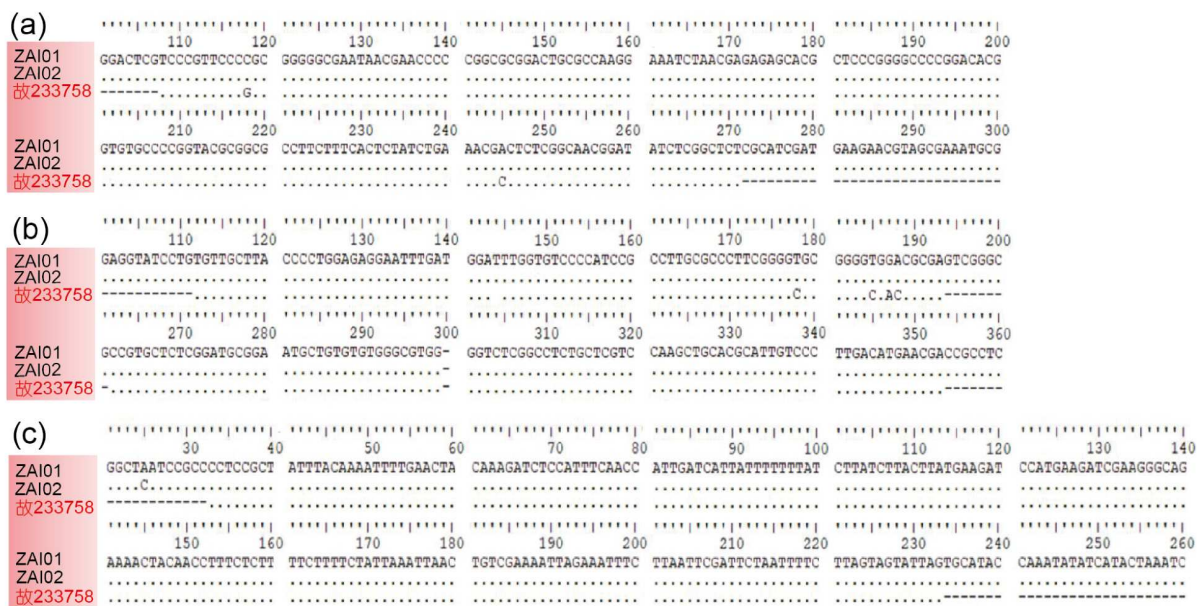


图5 清宫海桐皮(故233758)和椿叶花椒的ITS、ETS、*trnH-psbA*序列比对结果。(a) ITS; (b) ETS; (c) *trnH-psbA*
 Figure 5 The comparison results of ITS, ETS, and *trnH-psbA* sequences of “*Haitongnpi*” from imperial pharmacy in Qing Dynasty (GU233758) and *Zanthoxylum ailanthoides* Sieb. et. Zucc. (a) ITS; (b) ETS; (c) *trnH-psbA*

与ETS联合序列可以有效实现种间水平的鉴别^[66,67]。在清宫海桐皮古DNA中捕获到长度为164 bp ITS、172 bp ETS和201 bp *trnH-psbA*。通过建立ETS、*trnH-psbA*、ETS与ITS联合分子系统树,以及序列比对,从而实现了清宫海桐皮基原的准确鉴定。

在以往的植物考古学研究中,对未知种的植物遗存鉴定常使用植物形态学、孢粉学、淀粉粒或植硅体等证据^[75]。这些证据对植物类群鉴定的精准程度不一,一些鉴定结论往往不能尽如人意。本文综合运用了分子考古、有机残留物分析,共同推断出科属,进而运用花椒属的分子鉴别特征,采集现生样品,实现了基原的准确鉴定。本文建立了“科属-种”逐层鉴定的研究模式,为今后开展古代药材文物基原鉴定提供了借鉴。在未来的本草考古研究中,多种技术的相互印证,必将在药物遗存准确鉴定中发挥重要作用。

3.2 古代本草著作中关于海桐皮性状描述多节选自前人书籍,文献史料难以考证基原

古代本草著作对海桐皮的性状描述,不一定是作者自己亲自观察所述,有时引自前代本草。如宋《本草图经》(1061):“海桐皮,出南海已南山谷,今雷州及近海州郡亦有之。叶如手大,作三花尖。皮若梓白皮而

坚韧,可作绳,入水不烂”^[10]。其中“出南海已南山谷”“可作绳,入水不烂”来自宋《开宝本草》。《本草原始》(1612)^[16]：“始出南海已南山谷,今雷州及近海州郡亦有之。叶如手大,作三花尖。皮多刺,似桐皮,黄白色,故名海桐皮”。这些文字由前代数部本草杂糅而成。《本草汇言》(1624)^[76]描述:“苏氏曰:……叶大如手掌,有三尖。皮若梓皮,白而坚韧,可作绳,入水不烂。又有一种刺桐,生山谷中,文理细紧,而性喜折裂。体有巨刺,如欂櫨,其实如枫。又有一种棘桐皮,青叶圆大而长,高三四丈,开花红色,成朵而繁,为夏秋奇观。”这部分文字虽冠名为苏氏,其实主要引自《本草纲目》^[77]中“集解”项下“颂曰”内容,但又作了少许改动。而《本草纲目》“集解”项下“颂曰”内容,则由《本草图经》“海桐皮”和“桐”项下“棘桐”“刺桐”下内容杂糅而成。《本草详节》(1681)^[78]描述海桐皮:“文理细密,体有巨刺,实如枫子,叶圆大而长,高三四尺便有花,红色如火。”该段文字则《本草纲目》中引文剪裁而来。《本草述钩元》(1833)^[79]：“生南海及雷州近海州郡,叶如桐,其花附干而生,侧敷如掌,形若金凤,枝干有刺。刺桐生山谷中,纹理细紧,而性喜折裂,花繁如火,为夏秋荣观。(桐谱)”其中“侧敷如掌”“枝干有刺”引自《本草图经》“桐”条项下“岭南有刺桐,叶如梧桐,花侧敷

如掌,枝干有刺,花色深红”^[10]。“刺桐生山谷中,……为夏秋荣观”,该书标记引自《桐谱》。而《桐谱》内容则是将“刺桐”“棘桐”二者性状杂糅在一起^[80]。

纵观古代本草著作中对海桐皮的性状描述,后世本草引用前代本草,海桐皮的性状描述中糅合了“刺桐”“棘桐”等描述,有的本草著作标记了文献出处,有的则相互杂糅,未标记文献。明清时期几乎所有的本草著作中对海桐皮的描述,无一例外的都是这样。因此,根据本草著作对“海桐皮”性状,难以考证出真正的基原。

一些古代本草著作还绘有海桐皮图(图6),如宋《本草图经》“雷州海桐皮”,《本草品汇精要》《本草纲目》《本草原始》《本草汇言》《本草备要》等。其中《本草原始》是我国历史首部绘药材图谱的著作,所绘制的附图多为作者李中立根据实物绘制,栩栩如生。明清时期所有本草著作海桐皮的图,均绘出了“枝干有刺”的特征。《本草品汇精要》《本草纲目》《本草原始》图中叶片反映了“叶大如手掌,有三尖”的特征;而《本草备要》“海桐皮”图中完全抄袭于《本草汇言》,该图反映了“青叶圆大而长”的特征。不难看出,明清时期本草著作中海桐皮图多为作者根据文字描述所绘,而并非实际写生。

正是由于明清时期本草著作对“海桐皮”的描述及绘图,均为实际观察所致。因此,基于明清时期本草记

载对“海桐皮”开展的本草考证,难以考证其基原。在这种情况下,故宫博物院所珍藏的清宫御药房海桐皮药材文物,该实物史料对研究清代海桐皮的基原价值愈加显得珍贵。

3.3 清宫海桐皮药材文物为椿叶花椒*Zanthoxylum ailanthoides* Sieb. et. Zucc.作为海桐皮入药提供了实物证据

《本草品汇精要》(1505)^[14]:首次强调皮上有刺:“类梓白皮而有钉盖,擘之可脱”但是,仅凭该特征难以准确考证其基原。《本草图经》绘有“雷州海桐皮”图。但是该图信息有限,不同的学者考证结果不同,有的认为是木棉科植物木棉*Bombax malabaricum* DC.^[11](*Flora of China*修订为*Bombax ceiba* L.);有的认为与五加科植物刺楸*Kalopanax septemlobus* (Thunb.) Koidz.相似^[12,13]。《本草品汇精要》《本草纲目》《本草原始》《本草汇言》等著作也有图(图6)。这些图中树干均画出了较粗大的明显钉刺。但是依然难以考证到具体的科属。目前,纵观各位学者对海桐皮的本草考证,均未得出可能为芸香科植物椿叶花椒*Zanthoxylum ailanthoides* Sieb. et. Zucc.^[11-13,81]。《药材学》(1960)记载“海桐皮”来源于豆科植物刺桐*Erythrina indica* L.(*Flora of China*修订为*Erythrina variegata* L.)的干燥树皮,在附注中注明:“市场‘上海桐皮’的品种较为复杂,

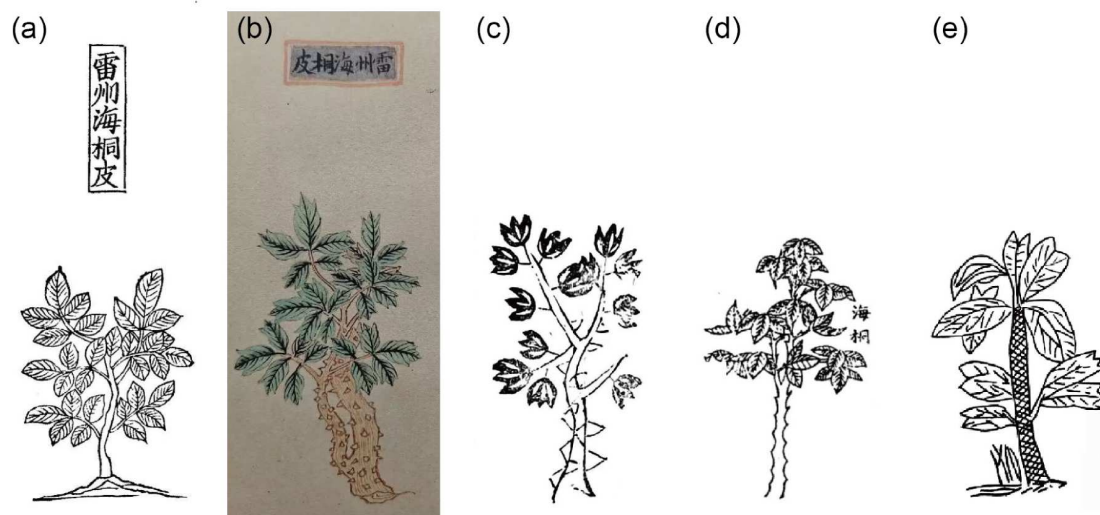


图6 历代本草海桐皮图版。(a)《本草图经》“雷州海桐皮”图;(b)《本草品汇精要》“海桐皮”图;(c)《本草纲目》“海桐皮”图;(d)《本草原始》“海桐皮”图;(e)《本草汇言》“海桐皮”图

Figure 6 Illustrations of “Haitongpi” in historical Chinese herbal texts. (a) Illustration of “Haitongpi” from *Bencao Tujin*; (b) illustration of “Haitongpi” from *Bencao Pinhui Jingyao*; (c) illustration of “Haitongpi” from *Compendium of Materia Medica*; (d) illustration of “Haitongpi” from *Bencao Huiyan*

据文献报道,尚有下列数种:芸香科植物食茱萸*Fagara ailanthoides* Engler的树皮...^[82]。1998年版《北京市中药材标准》记载“海桐皮”来源于椿叶花椒*Z. ailanthoides* Sieb. et. Zucc.或朵花椒*Z. molle* Rehd.的干燥树皮^[83]。本文对清宫御药房海桐皮药材文物的本草考古,为椿叶花椒*Z. ailanthoides*作清代宫廷海桐皮入

药提供了实物证据。

通过对故宫博物院现藏的清宫海桐皮药材文物进行本草考古研究,观一隅而知全貌,为研究清代海桐皮的药用历史提供依据。海桐皮始载于五代,药用历史悠久,其基原可能存在变迁,也可能因地域不同而不同。重构海桐皮的药用历史,尚需期待更多的史料依据。

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补充材料

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Summary for “清宫海桐皮本草考古：古DNA信息与化学特征的共同证据”

Bencao archaeology of “*Haitongpi*” from imperial pharmacy in Qing Dynasty: Common evidence based on ancient DNA information and chemical characteristics

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Bencao archaeology applies modern archaeological theories and methods to explore the intricate relationship between humans and medicinal substances, with a particular focus on unearthed drugs, medicinal sites, and related relics. This field aims to reconstruct the temporal and spatial frameworks of cultural remains and trace the historical evolution of Traditional Chinese Medicine (TCM). From ancient to modern times, the origin of medicinal materials has often undergone changes. A critical task in bencao archaeology is the accurate identification of the origins of medicinal relics. Conducting research on the origins of ancient medicinal materials can promote the inheritance and development of TCM, providing a foundation for ensuring clinical efficacy and safety. *Haitongpi*, first recorded in the *Haiyao Bencao* during the Five Dynasties period, has a long history of medicinal use. It was later recorded in the Qing Dynasty's *Yizong Jinjian*, where it was featured as a key ingredient in *Haitongpi* Decoction, used to dispel wind-dampness and alleviate pain. However, the precise botanical origin of *Haitongpi* is difficult to determine based solely on historical texts, as the modern medicinal use of this term encompasses plants from four families and six species distributed across different regions. The imperial palace, as the residence of the highest rulers in China's feudal society and the center of state power, maintained a meticulous standard for the selection and quality of medicines used in the Qing imperial pharmacy. Medicinal relics of *Haitongpi* preserved in the National Palace Museum provide crucial material evidence for exploring its historical usage and origin during the Qing Dynasty. In this study, a multidisciplinary approach combining molecular archaeology, and chemical profiling was employed to identify the origin of *Haitongpi* relics (GU233758) from the Qing imperial pharmacy. Initially, ancient DNA was extracted from the relics, and shotgun metagenomic sequencing was conducted, identifying the sample as belonging to the genus *Zanthoxylum*. Complementary chemical analysis using UPLC-Q-TOF-MS/MS revealed 20 characteristic compounds, including alkaloids, flavonoids, lignans, and coumarins, consistent with the chemical profile of *Zanthoxylum*. To further narrow down the species, a molecular phylogenetic tree was constructed using ITS, ETS, and *trnH-psbA* sequences. The results demonstrated a close genetic relationship between the *Haitongpi* sample and *Zanthoxylum ailanthoides* Sieb. et Zucc. By integrating morphological analysis, molecular techniques, and chemical characterization, this study established a systematic identification model for Qing Dynasty medicinal relics, progressing from family to genus to species. The findings confirmed that *Haitongpi* (GU233758) originated from the bark of *Z. ailanthoides*. This research not only provides a robust bencao archaeological foundation for tracing the origins of *Haitongpi* but also contributes to the preservation of Qing medical records and the inheritance of classic formulations. Moreover, it holds potential for improving clinical efficacy and medication safety through a deeper understanding of historical medicinal practices.

***Haitongpi*, Bencao archaeology, molecular archaeology, chemical characteristics**

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