

彝药乃斯补尼与其 4 种易混淆牛膝类药材的生药学鉴别研究

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摘要:目的 研究针对彝药乃斯补尼的准确鉴别方法,为其准确应用提供科学依据。方法 使用相机成像系统依据植物分类学进行基原差异识别,使用相机、体视显微镜及体式荧光显微镜成像系统综合应用建立性状鉴别方法,使用光学显微镜,荧光显微镜及偏光显微成像系统综合找出其显微鉴别(横切面及粉末)的差异,并针对乃斯补尼特有性成分建立其薄层鉴别方法。结果 建立了乃斯补尼药材与其 4 种混淆品基原、性状检索表,基原的主要区别点是花序的类型及着生方式、雄蕊的形态、叶片的颜色、根表皮的颜色及味道;性状鉴别的区别点为颜色、皮孔、断面、质地、气味及不同颜色光照下荧光反应的部位和强弱;显微鉴别中根横切面可通过三生维管束的圈数、中心维管束的股数及荧光反应进行区别;但粉末显微特征区别不明显。薄层色谱中以皂苷类成分川牛膝皂苷 A、川牛膝皂苷 B 及竹节参皂苷 IVa 对照品建立的薄层色谱能有效地区分乃斯补尼与其 4 种混淆品,以甾醇类成分杯苋甾酮对照品建立的薄层色谱可有效区分除川牛膝外的其他 3 种混淆品。结论 乃斯补尼与其 4 种混淆品可以通过原植物、性状、显微横切及薄层色谱进行鉴别,且 5 种药材成分差异明显,不宜混用。

关键词:彝药;乃斯补尼;鉴别;基原;性状;显微鉴别;薄层色谱

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Pharmacognostic Identification for Yi Medicine *Cyathulae Capitatae Radix* and Its Four Confusable *Achyranthes Medicinal Materials*

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ABSTRACT: OBJECTIVE To investigate accurate identification methods for the Yi medicine *Cyathulae Capitatae Radix*, and provide scientific basis for its accurate application. **METHODS** A camera imaging system was used to identify original plant differences based on plant taxonomy. A trait identification method was established through the comprehensive application of cameras, stereo-microscopes, and fluorescence microscopy imaging systems. Optical microscopy, fluorescence microscopy, and polarizing microscopy imaging systems were used to identify the differences in microscopic identification (cross section and powder), and a thin layer identification method was established for the unique components of *Cyathulae Capitatae Radix*. **RESULTS** A key table for the origin and characteristics of the *Cyathulae Capitatae Radix* and its four adulterants is established, the main differences of the origin are the type and mode of inflorescence, the shape of stamens, the color of leaves, and the color and taste of the root epidermis. The differences in characters identification include color, lenticels, cross-section, texture, odor, and the location and intensity of fluorescence response under different colored light conditions. In microscopic identification, the transverse section of the root can be distinguished by the number of circles in the three vascular bundles, the number of strands in the central vascular bundle, and fluorescence reaction, but the difference in powder microscopic characteristics is not significant. The thin layer chromatography established with reference materials of saponin components such as cyanoside A, cyanoside B and chikusetsu saponin IVa, in thin layer chromatography can effectively distinguish *Cyathulae Capitatae Radix* and its four adulterants, the thin layer chromatography established with the reference substance of sterol component amaranthone can effectively distinguish the other three adulterants except *Cyathulae Radix*. **CONCLUSION** *Cyathulae Capitatae Radix* and its four adulterants can be identified through their original plants, characteristics, microscopic cross-sections, and thin layer chromatography, and the five medicinal materials have significant differences in composition and should

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not be mixed.

KEY WORDS: Yi medicine; *Cyathulae Capitatae Radix*; identification; origin; characters; microscopic identification; thin layer chromatography

彝药乃斯补尼(彝文名:ꞑꞑꞑꞑ)为苋科植物头花杯苋[*Cyathula capitata* (Wall.) Moq.]的干燥根^[1],味苦、涩而略麻,性平^[2],有祛风除湿、祛瘀通经、强筋壮骨的功效^[3]。彝医临床常配伍其他药材使用,主要用于“斯色”病(痛风性关节炎),跌打损伤、风湿及类风湿等疾病。有学者^[4-5]考证发现以乃斯补尼为主药形成的彝医临床常用药方“我思”在治疗“斯色”病(痛风性关节炎)中具有治愈率高、复发小、无不良反应及标本兼治的优点。

《红河中草药》《中药志》《中国药典》(1963年版)^[6-8]中川牛膝项下,性状记载、植物图片和拉丁学名,均为乃斯补尼的基原植物头花杯苋,川牛膝是彝医治疗风湿类疾病的常用药,可能广泛存在使用品种为头花杯苋,但记载为川牛膝的情况。同时调研发现,牛膝类药材包括乃斯补尼、川牛膝、(怀)牛膝、柳叶牛膝、粗毛牛膝等多个品种,存在混用、同名异物以及同物异名等情况。其中,乃斯补尼当作川牛膝售卖的情况最为常见,故乃斯补尼常被当作川牛膝的主要伪品进行鉴别研究,但乃斯补尼在治疗风湿类疾病中有独特优势,亟须针对乃斯补尼建立准确的鉴别方法,为其准确应用提供科学依据。

查阅相关文献^[1,4-5],乃斯补尼仅应用于部分少数民族,国内外相关研究较少,目前尚无国家标准或地方标准收载乃斯补尼,而对于乃斯补尼、川牛膝、牛膝、粗毛牛膝及柳叶牛膝5种牛膝类药材的系统鉴别尚未见报道。本实验基于正伪品,多角度多仪器相结合研究其基原、性状、显微鉴别及薄层色谱之间的差异性,进行了多角度、深层次的分析,并建

立了相应的区分方式,为该药材的品种鉴别、质量控制及资源的有效利用提供依据。

1 材料

1.1 仪器

Revolution 正倒置一体电动化智能显微成像系统(北京深蓝云生物科技有限公司);SZX16 体视荧光显微镜,BX63 光学显微镜(日本 Olympus 公司);CAMAG TLC Visualizer 2 薄层色谱数码成像系统(瑞士 CAMAG 公司);硅胶 G 薄层板(青岛海洋化工厂);CPA225D 电子天平(德国 Sartorius 公司);Milli-Q 纯水系统(美国 Millipore 公司)。

1.2 药物与试剂

川牛膝皂苷 A 对照品(批号:110081-91-9,纯度 98.0%),川牛膝皂苷 B 对照品(批号:51161-58-1,纯度 98.5%),竹节参皂苷 IV a 对照品(批号:51415-02-2,纯度 95.0%)(成都普思生物科技股份有限公司);杯苋甾酮(批号:111804-201705,纯度 95.3%,中国食品药品检定研究院);水为 Milli-Q 纯化水。间苯三酚、盐酸、甲醇、正丁醇、乙酸乙酯、甲酸等(分析纯)。

乃斯补尼[*Cyathula capitata* (Wall.) Moq., 编号:M]、川牛膝(*Cyathula officinalis* Kuan, 编号:C)、牛膝(*Achyranthes bidentata* Blume, 编号:NX)、粗毛牛膝(*Achyranthes aspera* L., 编号:CM)及柳叶牛膝[*Achyranthes longifolia* (Makino) Makino, 编号:LY]药材样品经四川省药品检验研究院高必兴主管中药师鉴定。见表 1。

表 1 5 种牛膝类药材的样品信息

Tab. 1 Sample information of 5 five achyranthes medicinal materia

No.	Sample	Origin plant	Place of origin(in Chinese)	No.	Sample	Origin plant	Place of origin(in Chinese)
M1	<i>Cyathulae capitatae Radix</i>	<i>Cyathula capitata</i> (Wall.) Moq.	Yuzhong District Chongqing (重庆渝中区)	M10	<i>Cyathulae Capitatae Radix</i>	<i>Cyathula capitata</i> (Wall.) Moq.	Liangshan Sichuan (四川凉山)
M2	<i>Cyathulae capitatae Radix</i>	<i>Cyathula capitata</i> (Wall.) Moq.	Yuzhong District Chongqing (重庆渝中区)	C1	<i>Cyathulae Radix</i>	<i>Cyathula officinalis</i> Kuan	Enshi Hubei (湖北恩施)
M3	<i>Cyathulae capitatae Radix</i>	<i>Cyathula capitata</i> (Wall.) Moq.	Chengdu Sichuan (四川成都)	C2	<i>Cyathulae Radix</i>	<i>Cyathula officinalis</i> Kuan	Liangshan Sichuan (四川凉山)
M4	<i>Cyathulae capitatae Radix</i>	<i>Cyathula capitata</i> (Wall.) Moq.	Chengdu Sichuan (四川成都)	C3	<i>Cyathulae Radix</i>	<i>Cyathula officinalis</i> Kuan	Leshan Sichuan (四川乐山)
M5	<i>Cyathulae capitatae Radix</i>	<i>Cyathula capitata</i> (Wall.) Moq.	Shaoyang Hunan (湖南邵阳)	C4	<i>Cyathulae Radix</i>	<i>Cyathula officinalis</i> Kuan	Fengjie Chongqing (重庆奉节)
M6	<i>Cyathulae capitatae Radix</i>	<i>Cyathula capitata</i> (Wall.) Moq.	Kunming Yunnan (云南昆明)	C5	<i>Cyathulae Radix</i>	<i>Cyathula officinalis</i> Kuan	Ya'an Sichuan (四川雅安)
M7	<i>Cyathulae capitatae Radix</i>	<i>Cyathula capitata</i> (Wall.) Moq.	Dali Yunnan (云南大理)	NX	<i>Achyranthis Bidentatae Radix</i>	<i>Achyranthes bidentata</i> Blume	Yongzhou Hunan (湖南永州)
M8	<i>Cyathulae capitatae Radix</i>	<i>Cyathula capitata</i> (Wall.) Moq.	Xichang Sichuan (四川西昌)	CM	<i>Achyranthis Asperae Radix</i>	<i>Achyranthes aspera</i> L.	Liangshan Sichuan (四川凉山)
M9	<i>Cyathulae capitatae Radix</i>	<i>Cyathula capitata</i> (Wall.) Moq.	Xichang Sichuan (四川西昌)	LY	<i>Achyranthis Longifoliae Radix</i>	<i>Achyranthes longifolia</i> (Makino) Makino	Liangshan Sichuan (四川凉山)

2 方法与结果

2.1 基原鉴定

实地采集样本,使用相机及体视显微镜拍摄,比较并区分乃斯补尼、川牛膝、牛膝、粗毛牛膝和柳叶牛膝的植物形态。

通过观察原植物和查阅《中国植物志》,乃斯补尼、川牛膝、牛膝、粗毛牛膝和柳叶牛膝植物分类检索表如下。

1. 二歧聚伞花序顶生或腋生,密集成花团球。
 2. 叶片窄椭圆形;花团球有时近单生或穗状;花干后黑灰色;雄蕊顶端分裂明显;根灰褐色或棕红色,根条圆锥形,少扭曲,味苦、涩而略麻 …… 头花杯苋[*Cyathula capitata* (Wall.) Moq.]
 2. 叶片宽椭圆形,少数倒卵形;花团球常交互对生;花干后近白色;雄蕊顶端分裂不甚明显;根灰褐色或棕黄色,根条圆柱形,扭曲,味甘而黏,后微回苦 …… 川牛膝(*Cyathula officinalis* Kuan.)
1. 穗状花序顶生或腋生。
 3. 叶片上面深绿色,下面紫红色至深紫色;花序带紫红色,叶片披针形或宽披针形

…………… 柳叶牛膝[*Achyranthes longifolia* (Makino) Makino.]

3. 叶片下面不为紫红色。
 4. 叶片椭圆形或椭圆披针形,少数倒披针形;穗状花序仅顶生,小苞片顶端不弯曲,常带紫色;退化雄蕊顶端平截,流苏状长缘毛
 - …………… 粗毛牛膝(*Achyranthes aspera* L.)
 4. 叶片宽卵状倒卵形或椭圆状矩圆形;小苞片顶端弯曲,不带紫色;退化雄蕊顶端平圆,稍有缺刻状细锯齿,无流苏状长缘毛 …… 牛膝(*Achyranthes bidentata* Bl.)

5者之间的区别见表2~3及图1~4。结果表明,乃斯补尼的花为二歧聚伞花序,密集为花团球,多单生,根表皮为棕红色,味苦、涩而麻;川牛膝的花为二歧聚伞花序,密集为花团球,多数交互对生,在枝顶端呈穗状排列,密集,根表皮为棕黄色,味甘;柳叶牛膝为穗状花序,叶片下表面紫红色;粗毛牛膝为穗状花序,小苞片顶端不弯曲,常带紫色;牛膝为穗状花序,小苞片顶端弯曲,不带紫色。由此可以区分乃斯补尼及其混淆品。

表2 乃斯补尼及其混淆品基原鉴别特征

Tab. 2 Identification characteristics of the origins of *Cyathulae Capitatae Radix* and adulterants

Sample	Flower	Leaf	Root
<i>Cyathulae Capitatae Radix</i>	The flowers are densely integrated into flower clusters, with a diameter of 2–4 centimeters. They are nearly solitary or form short spike like inflorescences, and when dried, they are black gray. The apical division of the degenerated stamens is obvious	The leaves are papery, narrow elliptical in shape, with sparse villous growth on both sides and ciliate	Root gray brown or brownish red, conical, less twisted, bitter, astringent and slightly tingling in taste
<i>Cyathulae Radix</i>	The flowers are densely integrated into flower clusters, with a diameter of 1–1.5 centimeters. Most of them are opposite to each other on the inflorescence axis, arranged in spikes at the top of the branches, dense or 2–3 centimeters apart. After drying, they are nearly white, and the division of the degenerated stamen tip is not obvious	The leaves are wide elliptical in shape, with a few being obovate in shape. There are attached rough hairs on the top and dense hairs on the bottom	Root grayish brown or brownish yellow, cylindrical, twisted, sweet and sticky in taste, with a slightly bitter aftertaste
<i>Achyranthis Bidentatae Radix</i>	Spike inflorescence terminal or axillary, degenerated stamens with flat and round tips, slightly notched serrations, and no tassel like long cilia	Leaves broad, ovate, obovate, or elliptical rectangular, with attached or spreading pubescence on both sides	Root soil yellow, conical, slightly sweet and slightly bitter in taste
<i>Achyranthis Asperae Radix</i>	Spike inflorescence terminal, with degenerated stamens at the top that are flat and long fringe like hairs	Leaves elliptical or elliptical lanceolate, with a few inverted lanceolate, both surfaces covered with pubescence, or nearly hairless	Root soil yellow, cylindrical, slender, with a sweet and slightly bitter taste
<i>Achyranthis Longifoliae Radix</i>	Spike inflorescence terminal or axillary, with a purplish red color, degenerated stamen in a square shape, with inconspicuous teeth at the top	Leaves lanceolate or broadly lanceolate, with purplish red to deep purple underneath, sparsely covered with pubescence on both sides	Root light red to red, conical, slightly sweet taste

2.2 性状鉴别

选取典型、有代表性的样品放在白色背景台面上,使样品呈现出自然的状态或进行染色,放好标尺,对其进行观察,用数码相机进行拍摄,记录外观特征。运用相机成像系统、体视日光及荧光显微镜程序系统,分别在可见光及荧光下观察样品的表皮及断面,比较记录乃斯补尼、川牛膝、牛膝、粗毛牛膝及柳叶牛膝的性状特征。

五者之间的差别见表3及图5。结果表明,主要的差别在于根的颜色、皮孔、断面、质地、气味及不同颜色光照下产生荧光反应的部位及强弱。乃斯补尼

皮孔圆而凸起明显,断面质地为纤维状,GFP模式下异型维管束最为清晰,质地较脆,易于折断,味苦而麻;川牛膝皮孔横长,不凸起,断面略角质,在紫外(UV)、刺激红色荧光蛋白(GFPNB)、和刺激绿色荧光蛋白(DS-RED)模式下均较清晰,质韧,不易折断,味甜;牛膝具圆形凸起皮孔,断面角质状,UV模式下较为清晰,质地软,易折断,味微甜而稍苦涩;粗毛牛膝具圆形凸起皮孔,断面略纤维状,UV模式下较为清晰,质脆,易折断,味微甘而后稍苦涩;柳叶牛膝表面为浅红褐色,具圆形凸起皮孔,断面略纤维状,中心略呈紫红色,可见光下即可清晰观察到异型维管束。

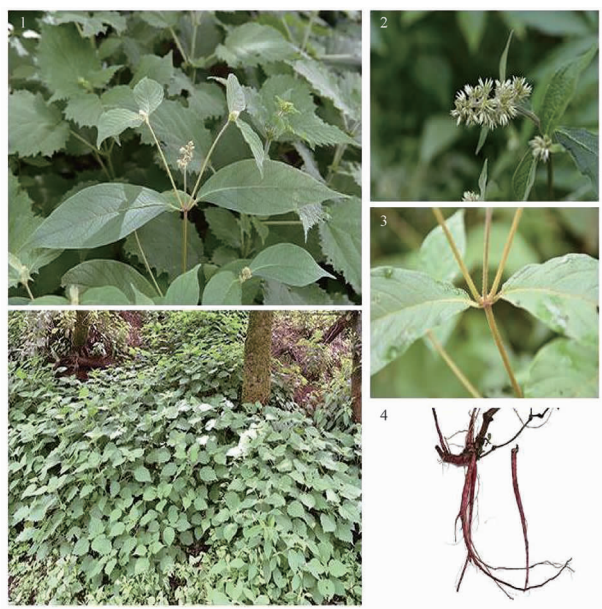
表3 乃斯补尼及其混淆品性状鉴别特征

Tab. 3 Identification characteristics of the characters of *Cyathulae Capitatae Radix* and adulterants

Variety	Colour	Lenticel	Section					Texture	Odour and taste	
			Texture	Stain	Visible light	UV	GFP			RFP
<i>Cyathulae Capitatae Radix</i>	Surface brownish yellow or brownish brown	The lenticel is round and has obvious protrusions	Fibrous	Several rounds of tertiary vascular bundles	Scattered white fascicular dots, unclear tertiary vascular bundles	Blue fluorescence, scattered fascicular points	Green fluorescence, several rounds of tertiary vascular bundles, relatively clear	Red fluorescence, several rounds of tertiary vascular bundles	Crisp texture, easy to break	Rich odour, bitter and numb taste
<i>Cyathulae Radix</i>	Surface yellow brown or gray brown	The lenticel is horizontally long and not protruding	Slightly keratinized	Several rounds of tertiary vascular bundles	Scattered white fascicular dots, unclear tertiary vascular bundles	Cyan fluorescence, several rounds of tertiary vascular bundles	Green fluorescence, several rounds of tertiary vascular bundles	Red fluorescence, several rounds of tertiary vascular bundles	Durable and not easily broken	Weak odour, sweet taste
<i>Bidentatae Radix</i>	Surface grayish yellow to light brown	Circular raised lenticel	Keratinized	Tertiary vascular bundle 2-3 rounds	Unclear tertiary vascular bundles	Light blue fluorescence, several rounds of tertiary vascular bundles	Green fluorescence, indistinct tertiary vascular bundles	Red fluorescence	Soft texture, easy to break	Weak odour, slightly sweet and slightly bitter taste
<i>Achyranthis Asperae Radix</i>	Surface gray brown	Circular raised lenticel	Slightly fibrous	The formation of tertiary vascular bundles into rings is not obvious	Scattered white fascicular dots, unclear tertiary vascular bundles	Dark blue fluorescence, several rounds of tertiary vascular bundles, clear and visible parenchyma	Green fluorescence, indistinct tertiary vascular bundles, visible partial parenchyma	Red fluorescence, unclear parenchyma	Crisp texture, easy to break	Weak odour with a slightly sweet taste, followed by a slight bitterness
<i>Achyranthis Longifoliae Radix</i>	Surface reddish brown	Circular raised lenticel with obvious cracks	Slightly fibrous	The tertiary vascular bundles are scattered into rings, with a slightly purplish red center	Purplish red, several rounds of tertiary vascular bundles	Dark blue fluorescence, several rounds of tertiary vascular bundles	Green fluorescence	Red fluorescence, several rounds of tertiary vascular bundles	Hard and brittle, easy to break	Weak odour, slightly sweet taste

注: GFP - 绿色荧光蛋白; RFP - 红色荧光蛋白。

Note: GFP - Green fluorescent protein; RFP - Red fluorescent protein.



1 - 原植物; 2 - 花特写; 3 - 节特写; 4 - 根特写; 5 - 生境。

1 - original plant; 2 - flower close-up; 3 - section close-up; 4 - root close-up; 5 - habitat.

图1 乃斯补尼生境及原植物的鉴别要点特写

Fig. 1 Habitat and original plant map and close up of key points for identification of *Cyathulae Capitatae Radix*



1 - 原植物; 2 - 花特写; 3 - 叶特写; 4 - 根特写; 5 - 生境。

1 - original plant; 2 - flower close-up; 3 - leaf close-up; 4 - root close-up; 5 - habitat.

图2 川牛膝生境及原植物的鉴别要点特写

Fig. 2 Habitat and original plant map and close up of key points for identification of *Cyathulae Radix*



1 - 原植物; 2 - 花特写; 3 - 节特写; 4 - 根特写; 5 - 生境。
1 - original plant; 2 - flower close-up; 3 - section close-up; 4 - root close-up; 5 - habitat.

图3 粗毛牛膝生境及原植物的鉴别要点特写
Fig. 3 Habitat and original plant map and close up of key points for identification of *Achyranthes Asperae Radix*



1 - 原植物; 2 - 叶特写。
1 - original plant; 2 - leaf close-up.

图4 柳叶牛膝原植物特征图
Fig. 4 Original plant map of *Achyranthes Longifoliae Radix*

2.3 显微鉴别

2.3.1 根横切 取药材干品, 浸泡, 徒手切片, 水合氯醛试液透化, 稀甘油试液装片, 置显微镜下观察, 拍照, 分别在可见光及不同波长荧光下观察样品的横切面, 记录各横切面显微特征。见表4及图6~7。

结果表明, 乃斯补尼根横切显微特征与川牛膝、牛膝、粗毛牛膝及柳叶牛膝在中心维管束分股数、三生维管束轮数及不同波长光照下荧光反应强弱上存在一定差异。乃斯补尼三生维管束为3~8圈, 中央次生构造维管束为2~6股原型, 蓝色及黄色荧光较弱; 川牛膝三生维管束为2~11圈, 中央次生构造维管束为2~9股原型, 所有荧光均较强; 牛膝三生维

管束为2~4圈, 中央次生构造维管束为2~3股原型, 蓝色荧光较弱; 粗毛牛膝三生维管束为3~6圈, 中央次生构造维管束为3~9股原型, 蓝色荧光较弱; 柳叶牛膝三生维管束为2~4圈, 且间隔较窄, 中央次生构造维管束为2~4股原型, 几乎无蓝色及黄色荧光。

2.3.2 粉末显微鉴别 取药材干品, 粉碎, 过5号筛, 挑取少许置载玻片上, 水合氯醛试液透化, 稀甘油试液装片, 置显微镜下观察, 拍照, 记录粉末显微特征。

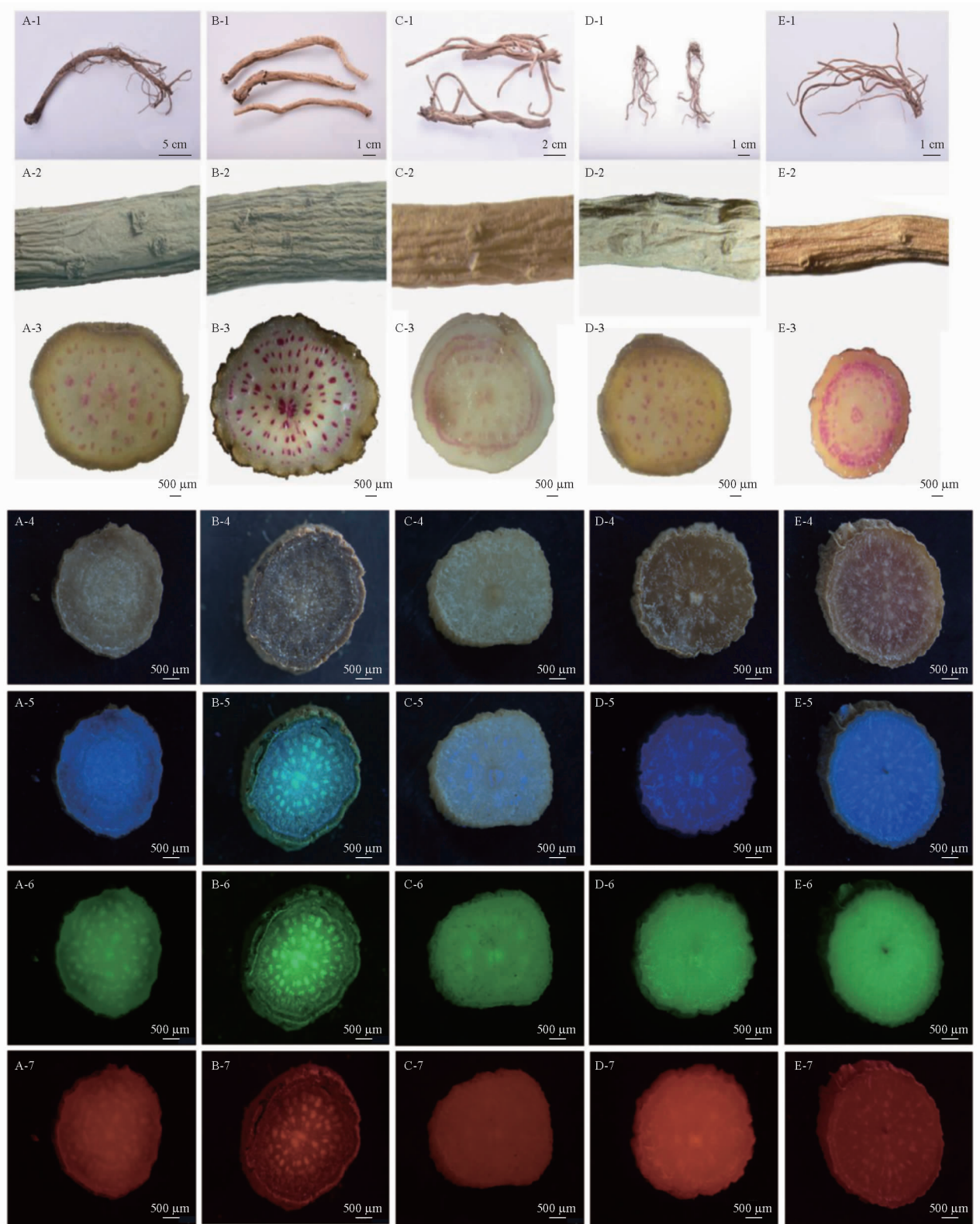
乃斯补尼粉末与川牛膝、牛膝、粗毛牛膝及柳叶牛膝在颜色、纤维直径及具缘纹孔导管直径上存在一定差异。但5种牛膝均含有草酸钙晶体、具缘纹孔导管、纤维及木栓细胞, 故川牛膝、牛膝、粗毛牛膝及柳叶牛膝的粉末鉴别与乃斯补尼并无明显区别。见表5及图8。

2.4 薄层色谱

2.4.1 皂苷类 取样品粉末0.2 g, 加体积分数80%甲醇50 mL, 加热回流30 min, 滤过, 用体积分数80%甲醇分3次(每次5 mL)清洗锥形瓶及漏斗, 取续滤液, 蒸干, 用水20 mL洗出, 水饱和正丁醇萃取3次, 每次20 mL, 蒸干, 残渣加甲醇使溶解, 转移至25 mL量瓶中, 并稀释至刻度, 摇匀, 滤过, 取续滤液作为供试品溶液。再取川牛膝皂苷A、川牛膝皂苷B及竹节参皂苷IVa对照品, 加甲醇制成每1 mL分别含川牛膝皂苷A、川牛膝皂苷B、竹节参皂苷IVa对照品0.2、0.2、0.1 mg的混合溶液, 作为对照品溶液。按照《中国药典》2020年版薄层色谱法(通则0502)试验, 取供试品溶液10 μL, 对照品溶液15 μL, 分别点于同一硅胶G薄层板上, 以乙酸乙酯-甲醇-水-甲酸(15:3:1:1)为展开剂, 展开, 取出, 晾干, 喷以10%硫酸乙醇试液, 在105 °C加热至斑点显色清晰, 置紫外光灯(365 nm)下检视。结果见图9。

结果表明, 乃斯补尼各批次样品溶液在与对照品色谱相应的位置上显相同颜色的荧光斑点, 而川牛膝、柳叶牛膝、牛膝、粗毛牛膝色谱图中, 均未检出3种对照品。

2.4.2 甾醇类 取样品粉末1.5 g, 加甲醇溶液10 mL, 超声处理60 min, 取续滤液, 作为供试品溶液。另取杯苋甾酮对照品, 加甲醇制成每1 mL含1 mg的溶液, 作为对照品溶液。按照《中国药典》2020年版薄层色谱法(通则0502)试验, 吸取供试品溶液10 μL, 对照品溶液5 μL, 分别点于同一



A - 乃斯补尼; B - 川牛膝; C - 牛膝; D - 粗毛牛膝; E - 柳叶牛膝; 1 - 药材; 2 - 表面特征; 3 - 断面(间苯三酚-盐酸染色); 4 - 断面(可见光下); 5 - 断面(UV); 6 - 断面(GFP); 7 - 断面(RFP)。

A - *Cyathulae Capitatae Radix*; B - *Cyathulae Radix*; C - *Achyranthis Bidentatae Radix*; D - *Achyranthis Asperae Radix*; E - *Achyranthis Longifoliae Radix*; 1 - medicinal materials; 2 - surface characteristics; 3 - cross section (stained with phloroglucinol and hydrochloric acid); 4 - cross section (under visible light); 5 - cross section (UV); 6 - cross section (GFP); 7 - cross section (RFP).

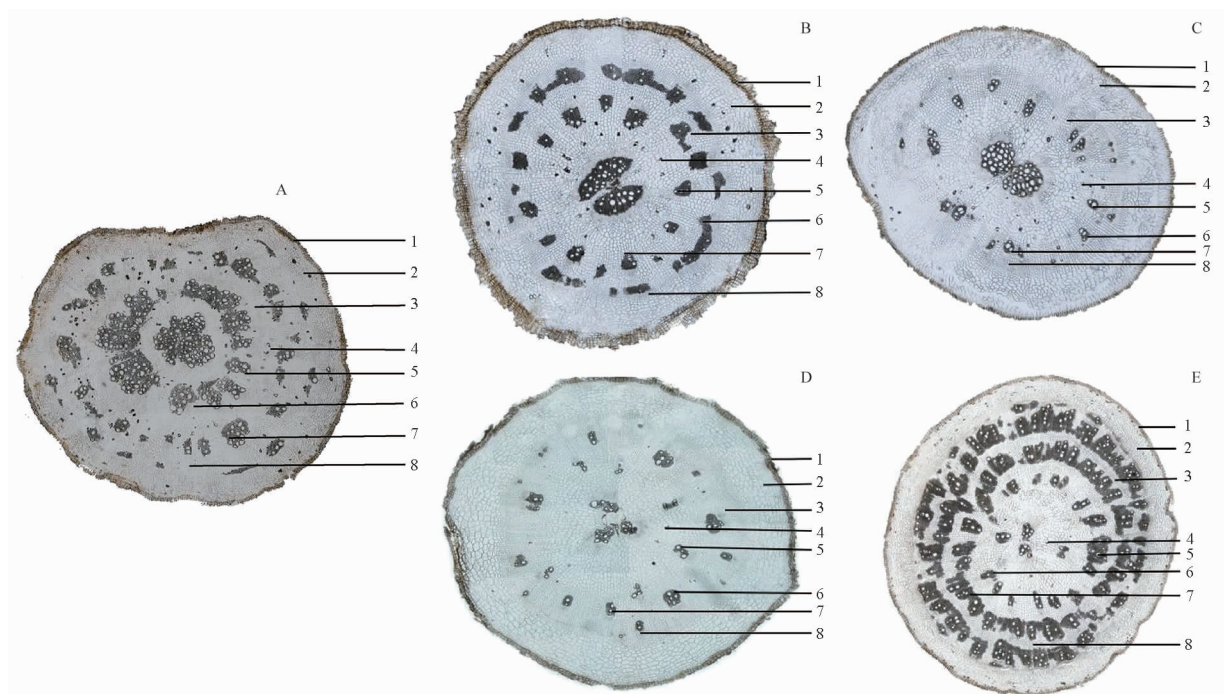
图5 乃斯补尼及其混淆品性状鉴别特征图

Fig. 5 Identification characteristics of characters of *Cyathulae Capitatae Radix* and adulterants

表 4 乃斯补尼及其混淆品根横切面显微特征主要区别

Tab. 4 Main differences in microscopic characteristics of root cross section of *Cyathulae Capitatae Radix* and adulterants

Identifying projects	<i>Cyathulae Capitatae Radix</i>	<i>Cyathulae Radix</i>	<i>Achyranthis Bidentatae Radix</i>	<i>Achyranthis Asperae Radix</i>	<i>Achyranthis Longifoliae Radix</i>
Number of vascular bundle rings	The tertiary vascular bundles are intermittently arranged in 3-8 circles	The tertiary vascular bundles are intermittently arranged in 2-11 circles	The tertiary vascular bundles are intermittently arranged in 2-4 circles	The tertiary vascular bundles are intermittently arranged in 3-6 circles	The tertiary vascular bundles are intermittently arranged in 2-4 circles, with narrow intervals between them
Central secondary vascular bundle	2-6 Strands	2-9 Strands	2-3 Strands	3-9 Strands	2-4 Strands
Fluorescence reaction	Blue fluorescence is weak with almost no yellow fluorescence	All fluorescence is strong	Blue fluorescence is weak	Blue fluorescence is extremely weak	Almost no blue and yellow fluorescence



A - 乃斯补尼; B - 川牛膝; C - 牛膝; D - 粗毛牛膝; E - 柳叶牛膝; 1 - 木栓层; 2 - 栓内层; 3 - 皮层; 4 - 草酸钙晶体; 5 - 导管; 6 - 纤维; 7 - 异型维管束; 8 - 韧皮部。
A - *cyathulae Capitatae Radix*; B - *cyathulae Radix*; C - *achyranthis Bidentatae Radix*; D - *achyranthis Asperae Radix*; E - *achyranthis Longifoliae Radix*; 1 - cork; 2 - phelloderm; 3 - cortex; 4 - calcium oxalate crystal; 5 - conduit; 6 - fiber; 7 - heterotypic vascular bundle; 8 - phloem.

图 6 乃斯补尼及其混淆品根的横切显微图

Fig. 6 Cross section micrograph of the roots of *Cyathulae Capitatae Radix* and adulterants

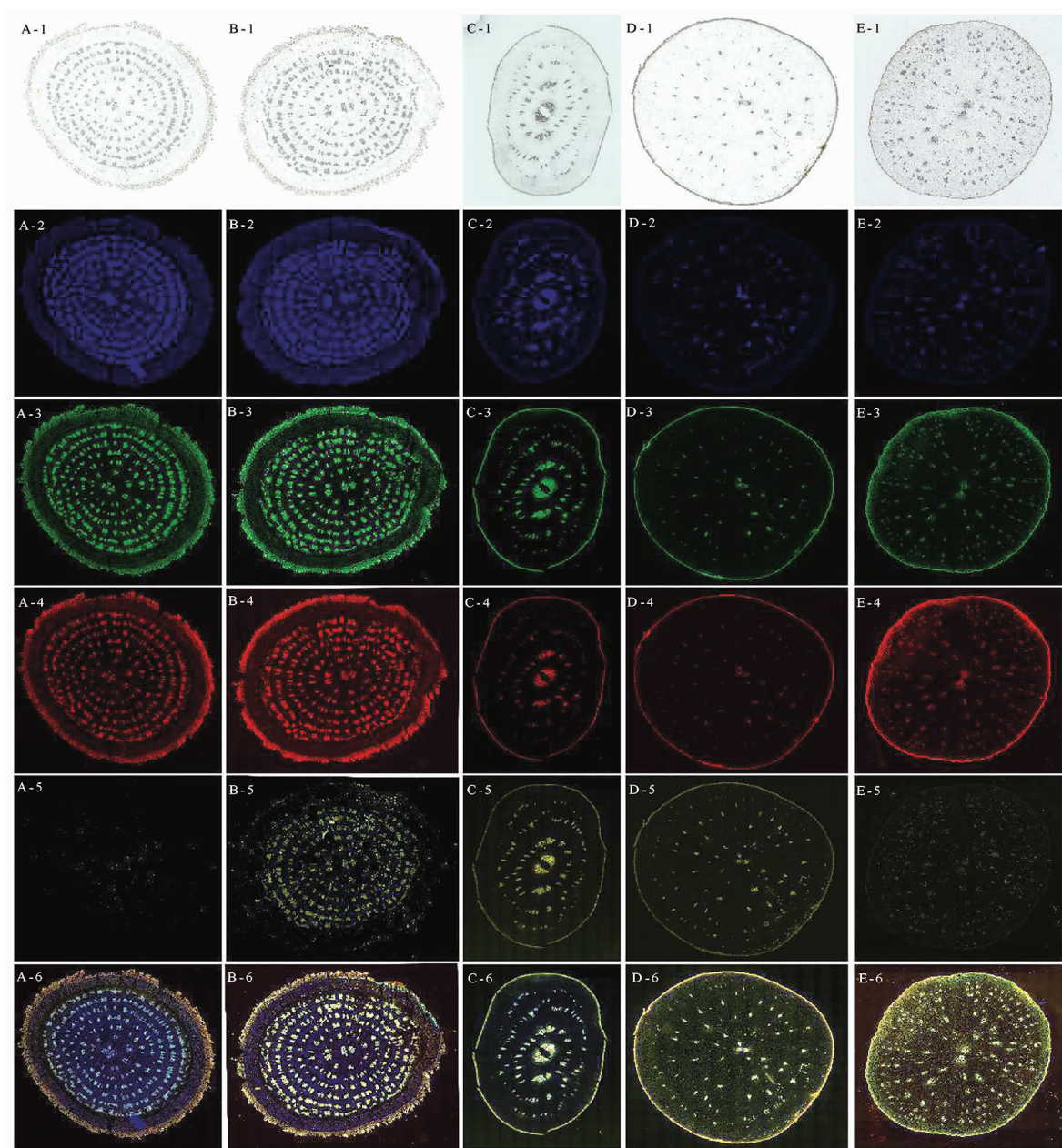
硅胶 G 薄层板上,以环己烷-乙酸乙酯-甲醇-冰醋酸 (3: 4: 1.5: 0.4) 为展开剂,展开,取出,晾干,喷以 10% 硫酸乙醇试液,在 105 °C 加热至斑点显色清晰,置紫外光灯 (365 nm) 下检视。结果见图 9。

结果表明,乃斯补尼与川牛膝各批次样品溶液在与对照品色谱相应的位置上显相同颜色的荧光斑点,而柳叶牛膝、牛膝、粗毛牛膝色谱图中,均未检出对照品。

3 讨论

本实验基于实时景深扩展-大图影像拼接技术,结合偏振光及荧光显微鉴别技术,对乃斯补尼及其 4 种混淆品进行了系统的生药学研究。乃斯补尼及其

混淆品分别来自于牛膝属及杯苋属,五者的区别主要在于原植物、药材性状及薄层色谱行为。其中乃斯补尼与川牛膝无论本草记载^[6-7],还是原植物形态、性状及显微上均具有极大的相似性,但经前期研究发现,乃斯补尼含有大量的皂苷类成分,为川牛膝所不具有的一大类成分,建立的方法能有效区分二者。乃斯补尼也含有少量的甾醇类成分,主要的甾醇类成分为杯苋甾酮,也为川牛膝《中国药典》2020 年版一部的质控成分^[9],其可作为乃斯补尼与粗毛牛膝、柳叶牛膝以及牛膝薄层鉴别的参考指标,但无法区分乃斯补尼及川牛膝。所有薄层色谱方法均参考文献^[10-11],考察了制样方法、展开系统、显色与检视方法、点样量及耐用性,以“2.4”项下所示方法为最佳。



A - 乃斯补尼; B - 川牛膝; C - 牛膝; D - 粗毛牛膝; E - 柳叶牛膝; 1 - 横切(可见光下); 2 - 横切(UV); 3 - 横切(GFP); 4 - 横切(RFP); 5 - 横切(YFP); 6 - 横切(2,3,4,5组合图)。

A - *cyathulae Capitatae Radix*; B - *cyathulae Radix*; C - *achyranthis Bidentatae Radix*; D - *achyranthis Asperae Radix*; E - *achyranthis Longifoliae Radix*. ; 1 - cross cutting (under visible light); 2 - cross cutting (UV); 3 - cross cutting (GFP); 4 - cross cutting (RFP); 5 - cross cutting (YFP); 6 - cross cutting (combination diagram of 2, 3, 4, and 5)

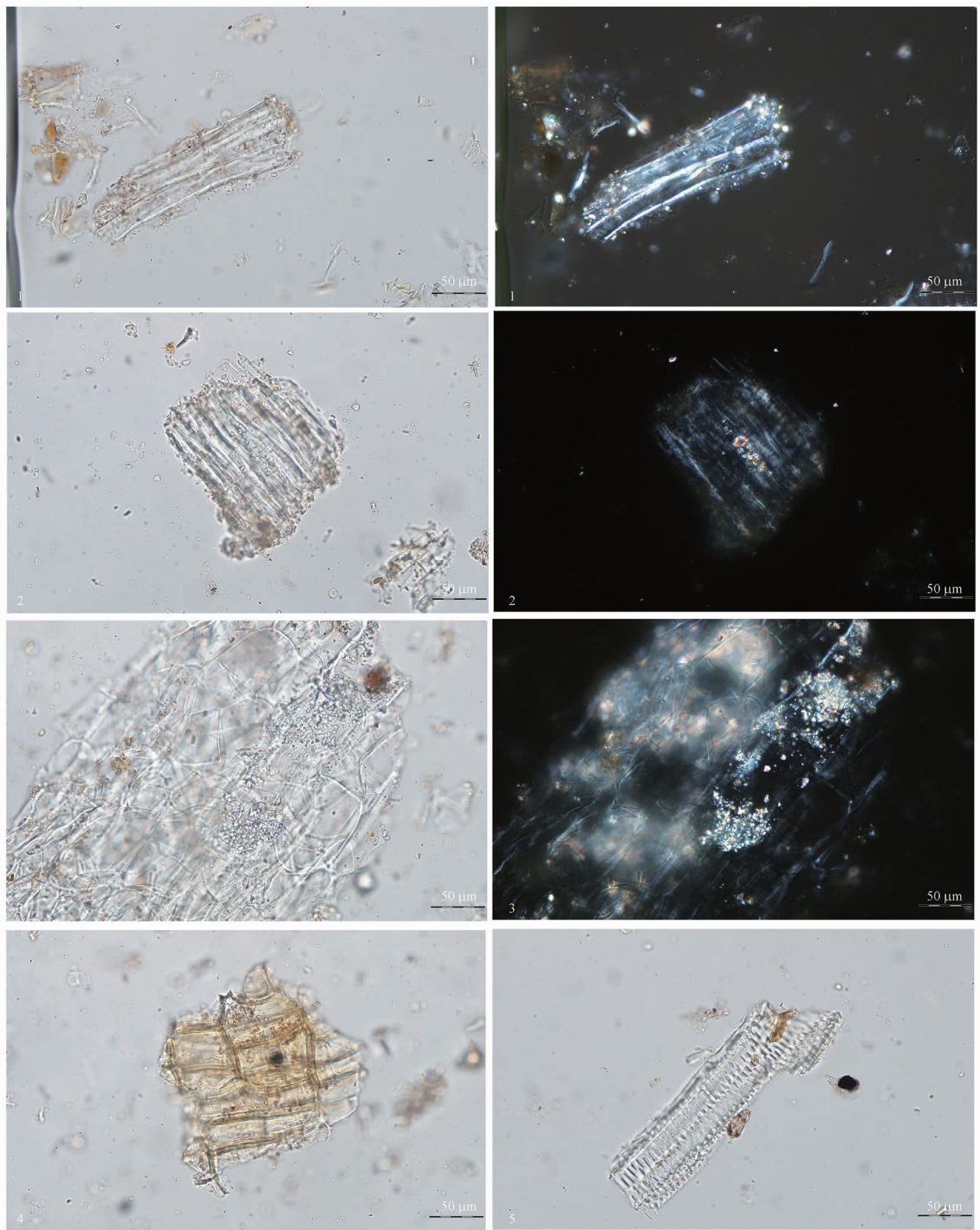
图7 乃斯补尼及其混淆品根的横切显微图

Fig. 7 Cross section micrograph of the roots of *Cyathulae Capitatae Radix* and adulterants

表5 乃斯补尼及其混淆品粉末显微特征主要区别

Tab. 5 Main differences in powder microscopic characteristics of *Cyathulae Capitatae Radix* and adulterants

Sample	Powder color	Fiber diameter/ μm	The diameter of bordered pit vessel/ μm
<i>Cyathulae Capitatae Radix</i>	Brown yellow or brown red	8 - 25	10 - 60
<i>Cyathulae Radix</i>	Yellowish-brown	8 - 25	10 - 80
<i>Achyranthis Bidentatae Radix</i>	Pale yellow	10 - 30	8 - 90
<i>Achyranthis Asperae Radix</i>	Yellowish-brown	11 - 25	10 - 80
<i>Achyranthis Longifoliae Radix</i>	Yellowish-brown	10 - 25	10 - 80



1 - 纤维; 2 - 草酸钙方晶; 3 - 草酸钙砂晶; 4 - 木栓细胞; 5 - 具缘纹孔导管。

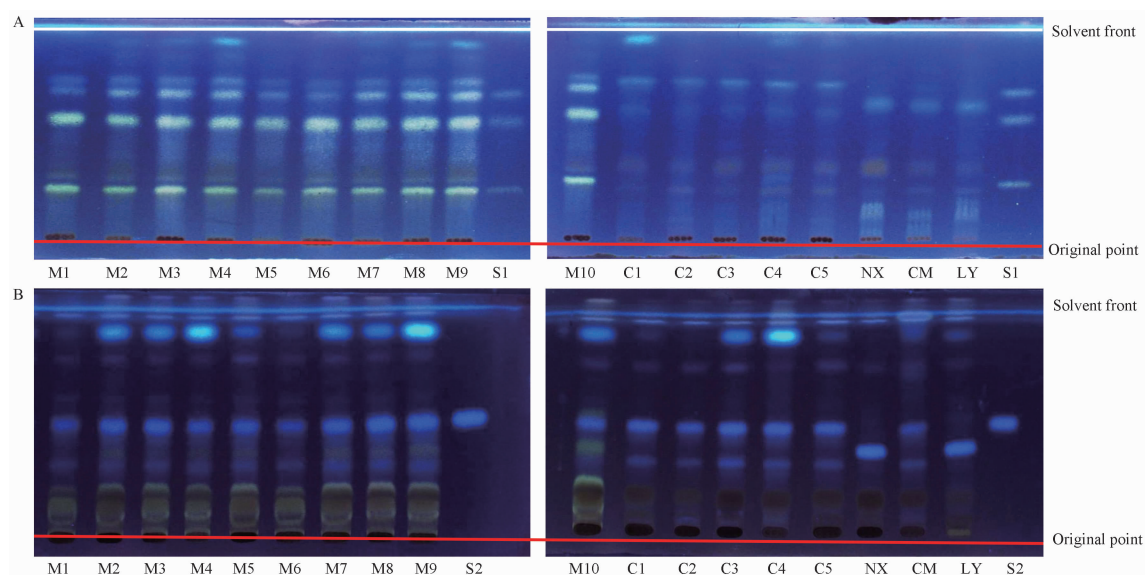
1 - fiber; 2 - calcium oxalate square crystal; 3 - calcium oxalate sand crystal; 4 - cork cell; 5 - bordered pit vessel.

图 8 乃斯补尼粉末显微特写图(×40)

Fig. 8 Microscopic features of *Cyathulae Capitatae Radix* powder(×40)

在乃斯补尼及其混淆品的性状特征探索中,药材表面特征易于观察,但具有一定的相似性,无法将上述药材完全区分,横断面需染色后才能较清晰的观察断面特征,操作较为繁琐,利用荧光体视显微镜同时考察了在可见光、紫外(UV)、刺激红色荧光蛋

白(RFP)和刺激绿色荧光蛋白(GFP)4个模式下的断面特征。观察乃斯补尼及其混淆品根横切显微特征时也同时考察了可见光、UV、RFP、GFP和刺激黄色荧光蛋白(YFP)5个模式下的特征。荧光图像具有不破坏或少破坏样品形态、试验用量少、快速、



A - 皂苷类; B - 甾酮类; M1 ~ M10, C1 ~ C5, NX, CM, LY - 样品; S1 - 川牛膝皂苷 A、川牛膝皂苷 B、竹节参皂苷 IVa 对照品 (自下而上); S2 - 杯苋甾酮对照品。
A - saponins; B - sterones; M1 - M10, C1 - C5, NX, CM, LY - samples; S1 - cyaonoside A, cyaonoside B and chikusetsu saponin IVa; S2 - amarantnone.

图9 乃斯补尼及其混淆品薄层色谱图

Fig. 9 Thin layer chromatogram of *Cyathulae Capitatae Radix* and adulterants

准确、环保等优点,且其有形态学特征,还具有荧光的颜色与强度的优势特征,二者结合更有助于准确鉴定^[12-13]。荧光的颜色与强度的不同也提示各牛膝类药材的组织结构及所含化学成分可能具有一定的区别,与药材性状研究、显微鉴定研究及薄层色谱研究结果相互印证。

乃斯补尼及其混淆品在成分上差异大,不应混用,否则影响临床使用的安全性及有效性,本实验建立了乃斯补尼的基原、性状、薄层色谱鉴别方法,并可与其混淆品川牛膝、牛膝、粗毛牛膝、柳叶牛膝有效区别开来,为乃斯补尼药用提供了质量控制和鉴别方法。

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