

Comparative Study on Leaf Morphology-Venation Pattern of *Hedyotis auricularia* L. and Its Adulterants

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Abstract [Objectives] To compare the leaf morphology and leaf venation patterns of *Hedyotis auricularia* L. and its adulterants, such as *Mitracarpus hirtus* (L.) DC., *Spermacoce pusilla* Wall. and *Spermacoce alata* Aubl., produced in Guangxi, so as to provide a simple and rapid identification method for the identification of *H. auricularia* L. in Guangxi. [Methods] LMVP (leaf morphology-venation pattern) identification method was used to study the characteristics of leaf morphology-venation pattern. [Results] Characteristics of leaf morphology-venation pattern: (i) *H. auricularia* L.: circular knotted-curved pinnate leaf venation without reaching the margin; the primary veins run straight without branches, the number of secondary veins is 5 to 9, and the angles included in tertiary veins are mostly near right angles or obtuse angles. (ii) *M. hirtus* (L.) DC.: curved pinnate leaf venation without reaching the margin; the primary veins run straight without obvious changes, the number of secondary veins is 3 to 5, and the tertiary veins are slender, and the included angles are mostly acute. (iii) *S. pusilla* Wall.: curved leaf venation without reaching the leaf margin; there are 4 to 5 pairs of secondary veins, most of which are opposite; the angle between the secondary veins and the primary veins in the middle and near the petiole is mostly medium acute angle, and the extension of the tertiary veins has no fixed direction. (iv) *S. alata* Aubl.: circular knotted-curved pinnate leaf venation; the primary veins are convex and straight without branches, and there are 4 to 6 pairs of secondary veins, alternating. The angle between secondary veins and primary veins is mostly medium acute angle, the angle between tertiary veins and secondary veins is near right angle, and the tertiary veins are mostly transversely extended. [Conclusions] The leaf morphology-venation patterns of the above-mentioned *H. auricularia* L. and *M. hirtus* (L.) DC., *S. pusilla* Wall., and *S. alata* Aubl. can be used as their identification features, and the identification method has certain operability, which provides an identification idea for the identification of Chinese herbal medicines.

Key words *Hedyotis auricularia* L., *Mitracarpus hirtus* (L.) DC., *Spermacoce pusilla* Wall., *Spermacoce alata* Aubl., Leaf venation pattern

1 Introduction

Hedyotis auricularia L. is a plant of the *Hedyotis* genus in the Rubiaceae family^[1]. The whole plant is used for medicinal purposes and it has the effects of clearing away heat and detoxifying, cooling blood and reducing swelling^[2]. It has good therapeutic effect on colds, sore throat, coughs, skin eczema and venomous snake bite^[3]. *H. auricularia* L. is also known as Jiyudancao, Xiyeyapochao and Jiejiefua in the *National Compilation of Chinese Herbal Medicines*, and it is bitter and cool in nature^[4]. It was first published in *Preparations for Raw Herbal Medicines* and there is another name Jiaojiancao in *Lingnan Medicine Collection Record* and it is recorded that this herb has opposite leaves, long tips, and splintery flowers, born from the leaf axils. According to this description, it is consistent with the characteristics of the current

Rubiaceae plant *H. auricularia* L.^[5–7] Modern studies have found that the chemical components of *H. auricularia* L. are mainly alkaloids, flavonoids, phenolic acids and terpenes^[8–10], which have antiviral, anti-inflammatory and anti-tumor effects^[11–16]. In Guangdong and Guangxi, due to the similar environment, many plants have similar external shapes and are difficult to distinguish, so easily confused varieties are prone to appear. *Mitracarpus hirtus* (L.) DC. is a plant of the Rubiaceae family, which is born on highway wasteland, mainly distributed in India, tropical South America, tropical East and West Africa, and distributed in Hainan, Guangdong, Guangxi and other places^[17]. According to recent studies, *M. hirtus* (L.) DC. is an invasive alien species^[18–22]. *Spermacoce pusilla* Wall. is a plant of the *Spermacoce* genus of the Rubiaceae family, also known as Jiasheshecao and Boliyacao. *Chinese Materia Medica* records that *Spermacoce pusilla* Wall. has a bitter taste and a cool nature, and has the effects of promoting blood circulation, removing blood stasis, reducing swelling and detoxification. It is mainly used to treat bruises, fractures, carbuncle swelling, snake bites and other symptoms^[23]. *Spermacoce alata* Aubl. is a plant of the *Spermacoce* genus of the Rubiaceae family, commonly known as Ribencao in Guangdong and Guangxi, and Zhushicao in Yunnan^[24]. *M. hirtus* (L.) DC., *S. pusilla* Wall., *S. alata* Aubl. and *H. auricularia* L. are both plants of the Rubiaceae family, with overlapping distribution areas and certain similarities in shape, as well as some differences^[25], which are prone to wrong picking and misuse. The leaf venation pattern identification method is suitable for fresh and dried leaves. Compared with the traditional identification meth-

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ods, the leaf venation pattern identification method is fast, simple, accurate and repeatable^[26–27]. Therefore, this paper conducts a comparative study of pharmacognosy from leaf morphology-venation pattern, which provides a reference for identification to ensure the accuracy and safety of clinical medication of *H. auricularia* L.

2 Materials and methods

2.1 Materials

2.1.1 Medicinal materials. The samples were collected from Wuming District, Nanning City, Guangxi, and were identified as the whole plant of *H. auricularia* L., *M. hirtus* (L.) DC. *S. pusilla* Wall. and *S. alata* Aubl. in the Rubiaceae family by associate professor Guo Min of the Department of Medicinal Botany of Guangxi University of Chinese Medicine.

2.1.2 Reagents. 95% ethanol (Guangxi Beilunhe Medical Industry Group Co., Ltd., Batch No.: 20211111), formalin (Sinopharm Group Chemical Reagents Co., Ltd., Batch No.: 20180508), glacial acetic acid (Sinopharm Group Chemical Reagent Co., Ltd., Batch No.: 20180702), chloral hydrate solution (Sinopharm Group Chemical Reagent Co., Ltd., Batch No.: 20181102).

2.1.3 Instruments. Electric blast drying oven (Shanghai Yiheng Scientific Instrument Co., Ltd., DHG9340A), paraffin slicer (Leica Instruments GmbH, Germany, model RM2235), high-speed pulverizer (Wenling Machinery Co., Ltd., model DFY-300C), fluorescence biomicroscope (Nanjing Hengqiao Instrument Co., Ltd., model Ni-U).

2.2 Methods For the observation of the leaf morphology of *H. auricularia* L., *M. hirtus* (L.) DC., *S. pusilla* Wall. and *S. alata* Aubl. in Guangxi, we refer to the General Principles for Identification of Medicinal Materials and Decoction Pieces of the Chinese Pharmacopoeia^[28].

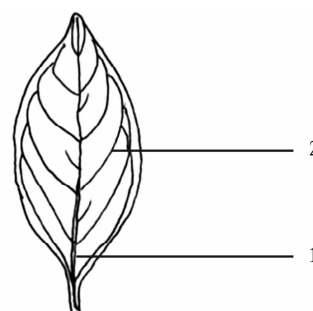
The leaf veins of the plants were irradiated with a multifunctional transmitter and then photographed with the macro camera function of the mobile phone. In addition, the leaf venation patterns of the two plants were observed with reference to the LMVP method^[29]. Vein preparation: Use alkaline test solution (such as 30% NaOH solution) to boil the leaves for about 40 min and then take them out to remove the mesophyll and the pigments contained, to get clear vein specimens after the leaves become completely transparent.

3 Results and analysis

3.1 Characteristics of leaf morphology-venation pattern of *H. auricularia* L.

3.1.1 Leaf morphology. The single leaves are opposite, lanceolate to elliptic, 3.5–8.0 cm long and 1.0–3.5 cm wide, with the widest part located in the middle. Leaf blade is flat, leaf tip is acuminate, leaf base is cuneate, leaf margin is entire, sometimes serrate. The upper surface of the leaves is green, the base of the middle vein is slightly convex, and the remaining veins are slightly concave. The lower surface of the leaves is light green with raised veins. The petiole is short and densely covered with brown-yellow

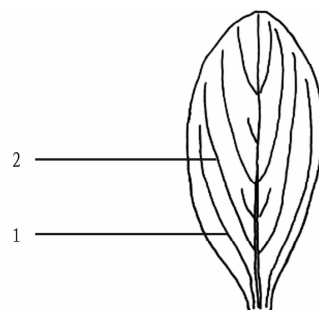
tiny hairs. Texture is leathery. The smell is slightly special, the taste is bitter and slightly astringent (Fig. 1).



NOTE 1. primary vein; 2. secondary vein.

Fig. 1 Leaf morphology and primary and secondary leaf venation pattern of *Hedyotis auricularia* L.

3.1.2 Leaf venation pattern. It is a circular knotted-curved pinnate leaf venation that does not reach the margin. The blade has only one primary vein, straight without branches, and some sections are slightly curved. The secondary veins curve upward and connect with each other at the tail to form an obvious vein ring. The vein ring connects with the adjacent secondary veins above at an acute angle. The number of veins on both sides is the same or different, with 5 to 9 veins, and the direction is relatively uniform. The arc shape increases its curvature smoothly or slowly, and the included angles are mostly medium acute angles and narrow acute angles. Secondary interstitial veins are occasionally seen, not obvious, and are simple. The tertiary veins and the primary veins have a vertical relationship, forming a right-angle network, with no branches, and the included angles are mostly near right angles or obtuse angles, arranged in parallel between the secondary veins (Fig. 2).



NOTE 1. primary vein; 2. secondary vein.

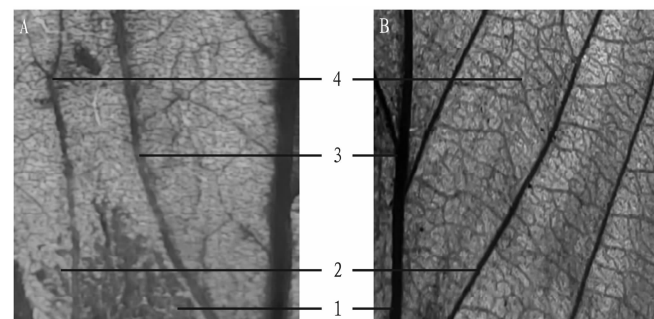
Fig. 2 Leaf morphology and primary and secondary leaf venation pattern of *Mitracarpus hirtus* (L.) DC.

3.2 Characteristics of leaf morphology-venation pattern of *Mitracarpus hirtus* (L.) DC.

3.2.1 Leaf morphology. Simple leaves are opposite, and apical leaves are clustered. The leaves are oblong to lanceolate, with the widest part in the middle, 1.7–3.5 cm long and 0.3–1.6 cm wide. The leaf blade is flat, the tip is short pointed, the leaf base is tapered, and the leaf margin is entire, sometimes slightly wavy. There is no petiole, with fine white hair on the leaf surface, the upper surface is brown-green, and the lower surface is light-col-

ored and dull.

The veins on the upper surface are slightly concave, and the veins on the lower surface are slightly convex (Fig. 3).



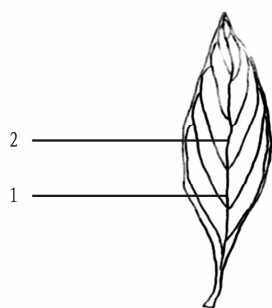
NOTE 1. primary vein; 2. secondary vein; 3. tertiary vein; 4. quaternary vein.

Fig. 3 Partial leaf venation pattern of *Hedyotis auricularia* L. (A) and *Mitracarpus hirtus* (L.) DC. (B)

3.2.2 Leaf venation pattern. It is curved pinnate leaf venation that does not reach the margin. There is only one primary vein, which goes straight and has no obvious change. The secondary veins curve upward, gradually tapering on the inner side of the leaf margin towards the leaf apex without forming an obvious vein ring. The number of veins on both sides is the same or different, with 3 to 5, mostly alternate, and the included angle is mostly narrow acute angle or middle acute angle, and the included angle at the base is narrow acute angle. Secondary interstitial veins are occasionally seen, not obvious, and are simple. The tertiary veins are slender, and the included angles are mostly acute.

3.3 Leaf morphology-venation pattern of *Spermacoce pusilla* Wall.

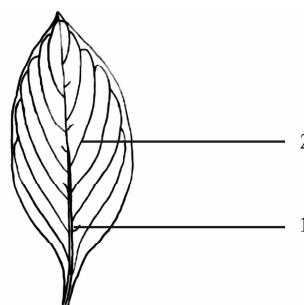
3.3.1 Leaf morphology. The leaves are single, with two opposite, two groups of adjacent leaves above and below are diagonally distributed, and the petioles are close to sessile; the stipules are very close to the petiole, with several long reddish-brown hairs on the top; the leaves are fusiform or lanceolate, narrow and long, 0.3–1.1 cm wide and 1.9–4.8 cm long. The leaves have no serrations, the apex is acuminate, the middle is the widest, and the base is gradually narrower and closes to the petiole, rough on both sides; old leaves are dark green, young leaves are tender green, and a few are purple.



NOTE 1. primary vein; 2. secondary vein.

Fig. 4 Leaf morphology and primary and secondary leaf venation pattern of *Spermacoce pusilla* Wall.

3.3.2 Leaf venation pattern. It can be seen from Fig. 5 that the leaf venation of *S. pusilla* Wall. is curved and does not reach the leaf margin. The primary veins of fresh leaves are thick and straight, and there are no redundant branches. There are 4 to 5 pairs of secondary veins that curve upward, mostly opposite and occasionally alternate. Compared with the primary veins, they are smaller in diameter and connect with the secondary veins on the upper adjacent side, but they do not reach the leaf margin, the angle between the secondary vein and the primary vein in the middle and near the petiole is mostly mid-acute, and the included angle at the top is a small acute angle. The angle between the tertiary veins and the secondary veins is more acute than that between the secondary veins and the primary veins. The extension of the tertiary veins has no fixed direction, and most of them are connected with the primary to quaternary veins to form meshes of different sizes and shapes. The included angle of the quaternary veins is obviously larger, nearly right angle, and they also form meshes like tertiary veins. The quinary veins are unclear.



NOTE 1. primary vein; 2. secondary vein.

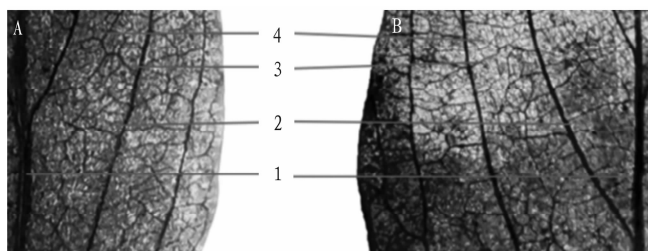
Fig. 5 Leaf morphology and primary and secondary leaf venation pattern of *Spermacoce alata* Aubl.

3.4 Leaf morphology-venation pattern of *Spermacoce alata* Aubl.

3.4.1 Leaf morphology. The leaves are simple, with two opposite, elliptical or ovate-round; the length is 2.0–6.3 cm, the width is 1.1–3.1 cm, the upper and lower surfaces have slight down, the upper surface is green, and the lower surface is light green; the leaf base is mostly crooked, the apex is sharp or obtuse, and the margin is entire; herbaceous.

3.4.2 Leaf venation pattern. It can be seen from Fig. 6 that the leaf venation of *S. alata* Aubl. is circular knotted-curved and pinnate. The primary veins of fresh leaves are thick and straight, and there are no redundant branches. There are 4–6 pairs of upward curved secondary veins, all of which are only alternate. Compared with the primary veins, they are smaller in diameter. They are connected with the upper adjacent secondary veins, but they do not reach the leaf margin. The angle between the secondary veins and the primary veins is mostly medium acute angle, and the angle at the top has not changed significantly. The angle between the tertiary veins and the secondary veins is nearly right angle. Tertiary veins are mostly extended horizontally, and at the same time, there is also extension without fixed direction and they connect with the primary to quaternary veins to form meshes of different sizes and shapes. The included angle of the quaternary veins is

mostly acute angle, and they also form meshes like tertiary veins. The quinary veins are unclear.



NOTE 1. primary vein; 2. secondary vein; 3. tertiary vein; 4. quaternary vein.

Fig.6 Partial leaf venation pattern of *Spermocoe pusilla* Wall. (A) and *Spermocoe alata* Aubl. (B)

4 Conclusions

(i) *H. auricularia* L.: The single leaves are opposite, broadly elliptical, with acuminate leaf apex, wedge-shaped base, short petiole; it is circular knotted-curved pinnate leaf venation that does not reach the margin. There is only one primary vein, straight without branches; the secondary veins curve upward and connect with each other at the tail to form an obvious vein ring, which connects the adjacent secondary veins above at an acute angle; the angle of tertiary veins is mostly near right angle or obtuse angle.

(ii) *M. hirtus* (L.) DC.: The single leaves are opposite, oblong, leaf tip is short and pointed, leaf bases are tapering, leaves are sessile; it is curved pinnate leaf venation that does not reach the margin. The primary veins run straight with no obvious change; the secondary veins curve upward, gradually tapering on the inner side of the leaf margin towards the leaf apex without forming an obvious vein ring; the tertiary veins are slender, and the included angles are mostly acute.

(iii) *S. pusilla* Wall.: Two groups of adjacent leaves at the top and bottom are diagonally distributed, and the leaves are fusiform or lanceolate, narrow and long, with the apex acuminate, and the base tapering; there are 4 to 5 pairs of secondary veins, mostly opposite and occasionally alternate; the angle between the secondary veins and the primary veins in the middle and near the petiole is mostly a medium acute angle, and the angle at the top is a small acute angle; there is no fixed direction for tertiary vein extension; the included angle of the quaternary veins is significantly larger.

(iv) *S. alata* Aubl.: The leaves are elliptical or ovate-round, with slight hairs on the upper and lower surfaces, the leaf base is mostly crooked, and the apex is sharp or obtuse; the primary veins are raised straight without branches, and there are 4 to 6 pairs of secondary veins, only alternating; the angle between the secondary veins and the primary veins is mostly medium acute angle, and the angle at the top has not changed obviously; the angle between the tertiary veins and the secondary veins is nearly right angle; tertiary veins are more commonly extended laterally.

5 Discussion

Traditional pharmacognosy identification usually includes basal

identification, trait identification, and microscopic identification. The identification of leaf morphology-venation pattern in this experiment is innovative to some extent. The morphological characteristics of plants in the same family are similar. Besides the traditional pharmacognosy identification methods, it is also a reliable identification method to distinguish different plants from the perspective of leaf morphology-venation pattern. However, the leaves of plants may change with the environment, or the leaf venation patterns of different seasons are different, which also has a certain impact on the identification of plants. Therefore, the study of leaf veins should focus on leaves in different environments and seasons.

The experimental results show that this method can provide basic research data for the identification basis and quality evaluation of *H. auricularia* L. and its adulterants—*M. hirtus* (L.) DC., *S. pusilla* Wall., *S. alata* Aubl. in Guangxi, provide scientific basis for development and utilization, and provide convenience for reasonable and standardized use, and ensure the safety of medication.

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