Microscopic Characteristics and Routine Detection of *Hedyotis hedyotidea* (DC.) Merr.

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Abstract [**Objectives**] To study the microscopic characteristics and routine detection of *Hedyotis hedyotidea* (DC.) Merr. [**Methods**] The microscopic characteristics of the root, stem cross section and the whole plant powder of *H. hedyotidea* were studied by the methods of slide-shaft section, biological staining and microscopic imaging; the routine detection items such as moisture, ash, acid-insoluble ash and extract were detected and analyzed in accordance with the requirements of *Chinese Pharmacopoeia*. [**Results**] The microscopic characteristics of the root, the stem and the whole plant powder of *H. hedyotidea* were obtained. The moisture content of 10 batches of *H. hedyotidea* samples was in the range of 4.25% -7.90%, the water-soluble extract is in the range of 15.08% -22.52%, the total ash was in the scope of 8.27% -10.45%, and the acid-insoluble ash was in 0.13% -0.95%. The proposed water content of *H. hedyotidea* should not exceed 10.00%, the water-soluble extract should not be less than 12.00%, the total ash content should not exceed 13.00%, and the acid-insoluble ash content should not exceed 1.20%. [**Conclusions**] The results of this experiment can provide a reference for the quality control and quality standard of *H. hedyotidea*.

Key words *Hedyotis hedyotidea* (DC.) Merr., Microscopic characteristics, Routine detection

1 Introduction

Hedyotis hedyotidea (DC.) Merr. is the dried whole plant of *Hedyotis* L. in family Rubiaceae Juss. It is distributed in Guangxi, Guangdong, Fujian, Taiwan, Guizhou, Yunnan and other places, and can be collected all year round[1]. Modern research shows that it has the effects of dispelling wind and dampness, renewing bones and muscles, clearing heat and relieving summer heat, and can be used for the treatment of heatstroke, cold and cough, gastroenteritis, rheumatoid arthritis, traumatic injury, fracture and skin eczema[1]. Modern pharmacological studies have shown that *H. hedyotidea* also has good analgesic and anti-inflammatory effects[2].

H. hedyotidea has rich resources and obvious efficacy. Besides, it is used as main raw materials of natural sweet plants in Zhuang people area together with leaves of *Momordica grosvenori*, *Lithocarpus polystachyus*, and *Rubus sachalinensis*, tender leaves of *Ampelopsis cantoniensis*, leaves of *Mycetia sinensis* (Hemsl.) Craib, leaves of *Glochidion philippicum* (Cav.) C. B. Rob., and *Scoparia dulcis* L. The root and stem of *H. hedyotidea* have sweet taste, which is used as a substitute for tea and medicine in Guangxi, and has the effects of clearing heat and moistening lung, detumescence and detoxification[3]. Therefore, *H. hedyotidea* can be widely used in pharmaceutical and food and beverage industries, and has great potential economic benefits. Therefore, we explored the microscopic characteristics and conventional detection of *H. hedyotidea*.

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2 Materials and methods

2.1 Materials

2.1.1 Instruments and reagents. Digital Microscope (BA210 Digital, Motic China Group, Ltd.); Semi-automatic microtome (RM2245, Leica Microsystems Shanghai Co., Ltd.) glass slide, cover glass. MOTIC IMAGES ADVANCED3.2 software; Semi-Automated Rotary Microtome (RM2245, Leica Microsystems Shanghai Co., Ltd.); Ultra Pure Water Machine (UPK-II, Chengdu UltraPure Technology Co., Ltd.); electric constant temperature water bath (WS70-1, Shanghai Shengxin Scientific Instrument Co., Ltd.); high-speed crusher (HWS-26, Shanghai Shengxin Scientific Instrument Co., Ltd.); electronic universal stove (YK-2000, Shandong Yikang Traditional Chinese Medicine Machinery Co., Ltd.); box-type resistance furnace (DL-1, Shanghai Keheng Industrial Development Co., Ltd.); blast drying oven (LX1811; Tianjin Laboratory Instrument Equipment Co., Ltd.); electronic balance (EL204, Mettler-Toledo Instruments (Shanghai) Co., Ltd.).

2.1.2 Reagents and medicinal materials. Formaldehyde (190704, Nanchang Yulu Experimental Equipment Co., Ltd.); anhydrous ethanol (2020071602; Sinopharm Chemical Reagent Co., Ltd.), glycerol (20190409; Sinopharm Chemical Reagent Co., Ltd.); chloral hydrate test solution (20220301; Shandong Puhuifen Chemical Technology Co., Ltd.), glacial acetic acid (20181205; Sinopharm Chemical Reagent Co., Ltd.); hydrochloric acid (2021010901, Chengdu Kelong Chemicals Co., Ltd.); silver nitrate (20036916, Sinopharm Chemical Reagent Co., Ltd.); pure water (laboratory-made). We collected 10 different places of ori-

gin H. hedyotidea samples from Guangxi, Guangdong, Fujian and other places. The sample information is listed in Table 1. All experimental samples were identified by associate professor Guo Min from Department of Medicinal Plants in Guangxi University of Chinese Medicine as the whole plant of *Hedyotis hedyotidea* (DC.) Merr.

Table 1 Sample information of *Hedyotis hedyotidea* (DC.) Merr.

No.	Production area	Collection time
NBT-1	Dadong Town, Bobai County, Yulin City, Guangxi	December 15, 2021
NBT-2	Bili Villa, Qingxiu District, Nanning City, Guangxi	December 20, 2021
NBT-3	Gaofeng Forest Farm, Yongwu Road, Xingning District, Nanning City, Guangxi	January 4, 2022
NBT-4	Pingji Town, Qinbei District, Qinzhou City, Guangxi	December 15, 2021
NBT-5	Shuitan Village, Liujiang District, Liuzhou City, Guangxi	December 15, 2021
NBT-6	Sanjiao Township, Jinxiu County, Jinxiu Town, Laibin City, Guangxi	December 16, 2021
NBT-7	Xinping Village, Guancheng Town, Pingnan County, Guigang City, Guangxi	December 18, 2021
NBT-8	Pingjiang Village, Longmen Town, Pubei County, Qinzhou City, Guangxi	December 15, 2021
NBT-9	Tangba Village, Longtan Town, Jiexi County, Jieyang City, Guangdong	December 15, 2021
NBT-10	Gukeng Village, Tingxi Town, Tongan District, Xiamen City, Fujian Province	December 18, 2021

2.2 Methods

2.2.1 Microscopic identification. (i) Cross section identification. The roots and stems of fresh *H. hedyotidea* samples were cut into 1 cm segments and fixed in FAA fixative for 1 day. The embedded sections were taken out and observed with coverslips.

(ii) Microscopic identification of the powder. Medicinal material processing: cutting the whole herb sample of *H. hedyotidea* into pieces, dried in an oven at 55 ℃, crushed, and screened with a No. 6 sieve for later use.

(iii) Slice preparation: took a proper amount of *H. hedyotidea* powder on a glass slide, added 2 – 3 drops of chloral hydrate solution, heated for permeabilization with an alcohol lamp, added 1 – 2 drops of dilute glycerin, loaded a cover glass, removed excess liquid after cooling, and observed under a microscope.

2.2.2 Routine detection. (i) Preparation of medicinal powder. The *H. hedyotidea* medicinal materials collected in Table 1 were cleaned, cut into small sections, dried in an oven at 55 ℃, crushed with a crusher, screened with a No. 7 sieve, mixed uniformly, packaged in a sealed bag, labeled, and sealed for later use.

(ii) Determination of moisture content. Moisture shall be determined according to the drying method in General Rule 0832 of Volume IV of *Chinese Pharmacopoeia* (Edition 2020)[2]. Took 2 g of *H. hedyotidea* powder in Section **2.2.1**, weighed it accurately, made 3 parallel samples for each batch, and spread them in a flat weighing bottle dried to constant weight, with a thickness of no more than 5 mm. Opened the bottle cap and dried it at 100 – 105 ℃ for 5 h, covered the bottle cap, transferred it to the dryer, cooled it for 30 min, weighed it accurately, dried it at the above temperature for 1 h, cooled it and weighed it until the difference between two consecutive weighing results does not exceed 5 mg. Based on the weight lost, the moisture content in the sample (%) was calculated.

(iii) Determination of water soluble extract. Water soluble extract shall be determined according to the extract determination method in General Rule 2201 of Volume IV of *Chinese Pharmacopoeia* (Edition 2020)[4]. Considering the actual situation, the hot soaking method can save time and has high extraction efficiency, so the hot soaking method was used to determine the extract; through comparing the content of 30%, 50%, 70% and 95% ethanol-soluble extract with that of water soluble extract, it was found that the content of water soluble extract was the highest, so pure water was selected as the solvent for the determination of extract content in this experiment. Took about 2 g of *H. hedyotidea* powder, weighed it accurately, made 3 pieces of each batch of samples in parallel, put it in a 100 mL Erlenmeyer flask, added 50 mL of pure water precisely, weighed it, let it stand for 1 h, heated it to boiling, and kept it slightly boiling for 1 h; after cooling, removed the conical flask, sealed the stopper, weighed the flask after cooling, made up the lost weight with pure water, shook up, filtered, precisely measured 25 mL of filtrate, put it into an evaporating dish that has been dried to constant weight, evaporated it in a water bath, dried it in an oven at 105 ℃ for 3 h, cooled it in a dryer for 30 min, and quickly and precisely weighed it. Finally, we calculated the content (%) of water-soluble extract in the sample based on the dried product.

(iv) Determination of total ash. The total ash was determined according to the acid-insoluble ash determination method in General Rule 2302 of Volume 4 of *Chinese Pharmacopoeia* (2020 Edition)[4]. Took 3 g of *H. hedyotidea* powder, weighed it accurately, made 3 parts of each batch of medicinal materials in parallel, put it into a crucible with constant weight, weighed it, put it into a muffle furnace to heat it slowly to avoid combustion, when the sample was completely carbonized, gradually raised the temperature to 500 – 600 ℃ to make it completely incinerated and reach constant weight, cooled it down and took it out of the dryer, calculated the total ash content (%) of the sample according to the weight of the residue.

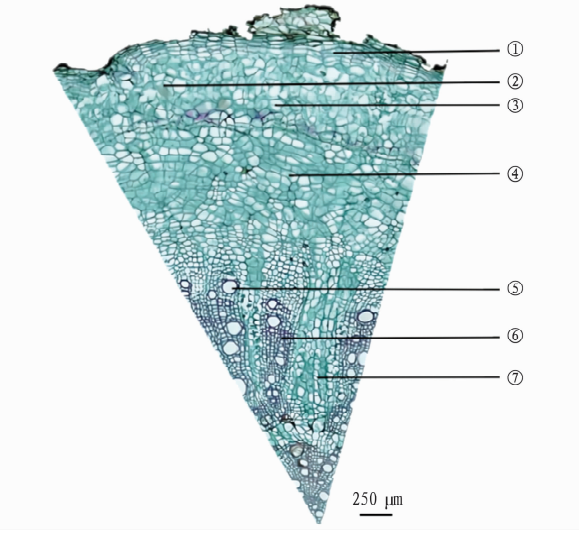
(v) Determination of acid-insoluble ash. Acid-insoluble ash was determined according to the determination method of acid-insoluble ash in General Rule 2302 of Volume 4 of *Chinese Pharmacopoeia* (2020 Edition)[4]. Took the ash obtained from the determination of total ash, carefully added about 10 mL of dilute hydrochloric acid into the crucible, covered the crucible with a watch glass, heated it in a water bath for 10 min, washed the watch glass with 5 mL of hot water, merged the washing solution into the cruci-

ble, filtered it with ashless filter paper, washed the residue in the crucible on the filter paper with water, and washed it until the washing solution did not show chloride reaction. The filter residue and the filter paper were transferred to the same crucible, dried and ignited to constant weight. The content of acid-insoluble ash (%) in the sample was calculated based on the weight of the residue.

3 Results and analysis

3.1 Results of microscopic identification experiment

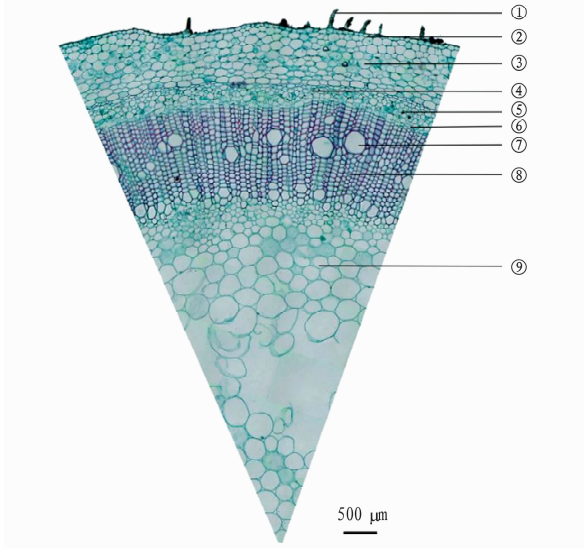
3.1.1 Microscopic characteristics of root cross section. The phellem of the cross section of the root was extended tangentially by 2 to 4 rows of cells, which were rectangular and closely arranged; several rows of cells in the cortex were round or oval, and some of them contained calcium oxalate needle crystals. The abnormal vascular bundles were of the ectophloem type, which were arranged in concentric rings and separated from each other by the connective tissue dominated by parenchyma cells. The phloem cells were polygonal and closely arranged. The xylem was wide and mainly composed of vessels and wood fibers. Most of the vessels were single and occasionally three vessels were scattered. The wood fibers were arranged neatly in the radial direction. The rays were obvious and composed of 20 – 25 rows of cells (Fig. 1).



Note: ① Phellem; ② Needle crystal of calcium oxalate; ③ Cortex; ④ Phloem; ⑤ Vessel; ⑥ Xylem; ⑦ Xylem ray.

Fig. 1 Cross section of root of *Hedyotis hedyotidea* (DC.) Merr.

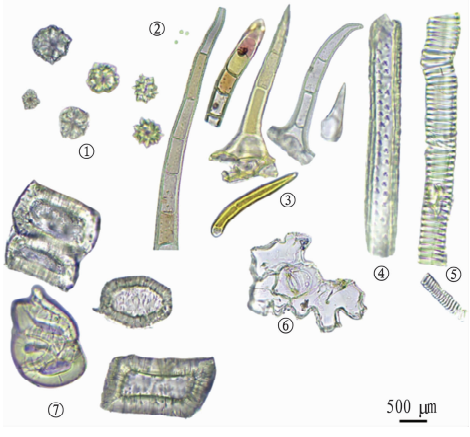
3.1.2 Microscopic characteristics of stem cross section. The epidermis of the stem cross section was composed of a row of regularly arranged quadrate cells, covered with a thin layer of cuticle, with non-glandular hairs. The cortex was composed of several layers of oval parenchyma cells, which were loosely arranged and have intercellular spaces, and the cells contain calcium oxalate cluster crystals and needle bundles. The endodermis was distinct and consisted of a row of cells with Casparian strip. The phloem was composed of polygonal parenchyma cells, which were closely arranged in a circular array; the cambium was not obvious, the xylem was arranged in a circular array, and the vessels were polygonal in transverse section and were scattered individually: wood fiber was polygonal or square-like; The xylem ray was not obvious, the cells were small, oval or square, 1 to 2 rows were closely arranged in the radial direction, and lignified; the pith occupied 2/3 of the transverse section of the stem, and the cells were large, round or oval, loosely arranged, with intercellular spaces (Fig. 2).



Note: ① Non-glandular hairs; ② Epidermis; ③ Cortex; ④ Calcium oxalate crystals; ⑤ Phloem; ⑥ Cambium; ⑦ Vessels; ⑧ Xylem; ⑨ Pith.

Fig. 2 Cross section of stem of *Hedyotis hedyotidea* (DC.) Merr.

3.1.3 Microscopic characteristics of the whole herb powder. The powder was light green, the surface of cork cell was polygonal, the starch was round, oval, *etc.*, mostly single grain, the umbilicus was punctate or slit-like, and the lamina was not obvious; there were two types of vessels: the threaded vessel and the pitted vessel. The diameter of the threaded vessel was 4 – 35 μm, and the diameter of the pitted vessel was 6 – 57 μm; the length of non-glandular hairs was 76 – 450 μm, and the number of cells was



Note: ① Calcium oxalate cluster crystals; ② Starch grains; ③ Non-glandular hairs; ④ Bordered pit vessels; ⑤ Spiral vessels; ⑥ Adventitious stomata; ⑦ Stone cell.

Fig. 3 Microstructure

1 – 9; calcium oxalate cluster crystals were contained in parenchyma cells, and the diameter of calcium oxalate cluster crystals was 4 – 33 μm; stone cells were scattered individually or in groups of 2 to 3, light yellow, rectangular, oval, triangle-like or square-like, 30 – 270 μm in diameter, with obvious pore grooves and laminae, thick walls and narrow cavities (Fig. 3).

3.2 Results of routine detection

3.2.1 Results of moisture content determination. The results are shown in Table 2. The moisture content of *H. hedyotidea* samples from different producing areas ranged from 4.25% to 7.90%, and the average value was 5.85%. Among the 10 batches of *H. hedyotidea* samples, the lowest water content was 4.29% in the sample from Yulin City (Bobai County), Guangxi, numbered NBT-1, and the highest water content was 7.89% in the sample (NBT-9) from Jieyang City, Guangdong.

3.2.2 Results of determination of water-soluble extract. The results are shown in Table 2. The water-soluble extract of *H. hedyotidea* samples from different producing areas ranged from 415.08% to 22.52%, and the average value was 19.48%. Among the 10 batches of *H. hedyotidea* samples from the producing areas, the sample from Guigang City, Guangxi (Pingnan County) with the number of NBT-7 had the highest content of 22.18%, and the sample from Laibin City, Guangxi (Jinxiu Town) with the number of NBT-6 had the lowest content of 15.21%.

3.2.3 Results of determination of total ash. The results are shown in Table 2. The total ash of *H. hedyotidea* samples from different producing areas ranged from 8.27% to 10.45%, and the average value was 9.01%. Among the 10 batches of *H. hedyotidea* samples from the producing areas, the sample from Qinzhou City, Guangxi (Pubei County) with the number of NBT-8 had the lowest total ash of 8.27%, and the sample from Guigang City, Guangxi (Guancheng County) with the number of NBT-7 had the highest total ash of 10.45%.

3.2.4 Results of determination of acid-insoluble ash. As shown in Table 2, the acid-insoluble ash of *H. hedyotidea* samples from different producing areas was in the range of 0.13% – 0.95%, and the average value was 0.39%. Among the 10 batches of *H. hedyotidea* samples from the producing areas, the sample from Qinzhou City, Guangxi (Pingjiang Village, Longmenxian Town, Pubei County) with the number of NBT-8 had the lowest acid-insoluble ash of 0.13%, and the sample from Nanning City, Guangxi (Bili Village) with the number of NBT-2 had the highest acid-insoluble ash of 0.95%.

Table 2 Routine detection results of 10 batches of *Hedyotis hedyotidea* (DC.) Merr. samples ($\bar{x} \pm s$, $n = 3$, %)

No.	Moisture content	Total ash	Acid-insoluble ash	Water-soluble extract
NBT-1	4.29 ± 0.04	8.37 ± 0.05	0.36 ± 0.01	16.20 ± 0.31
NBT-2	6.02 ± 0.11	9.10 ± 0.16	0.95 ± 0.01	19.72 ± 0.34
NBT-3	6.40 ± 0.07	8.95 ± 0.13	0.51 ± 0.01	21.65 ± 0.05
NBT-4	6.33 ± 0.10	8.41 ± 0.24	0.24 ± 0.01	21.69 ± 0.18
NBT-5	5.65 ± 0.13	8.51 ± 0.02	0.38 ± 0.00	16.55 ± 0.38
NBT-6	4.97 ± 0.08	8.44 ± 0.18	0.24 ± 0.01	15.21 ± 0.11
NBT-7	6.03 ± 0.04	10.45 ± 0.18	0.28 ± 0.01	22.18 ± 0.34
NBT-8	5.82 ± 0.10	8.27 ± 0.05	0.13 ± 0.00	20.81 ± 0.13
NBT-9	7.86 ± 0.15	9.39 ± 0.11	0.73 ± 0.02	20.43 ± 0.21
NBT-10	5.26 ± 0.09	10.25 ± 0.16	0.39 ± 0.01	20.38 ± 0.23

4 Conclusions and discussion

The study on microscopic characteristics of root, stem and whole herb powder of *H. hedyotidea* can provide technical support for distinguishing *H. hedyotidea* from other similar species, and can provide theoretical basis for the identification of *H. hedyotidea*. Routine detection of 10 batches of *H. hedyotidea* samples showed that the moisture content was in the range of 4.25% to 7.90%, the water-soluble extract was in the range of 15.08% to 22.52%, the total ash was in the range of 8.27% – 10.45%, and the acid-insoluble ash was in the range of 0.13% – 0.95%. Therefore, we proposed that *H. hedyotidea* has a moisture content of not more than 10.00%, a water-soluble extract of not less than 12.00%, a total ash content of not more than 13.00%, and an acid-insoluble ash of not less than 1.20%. This experiment can provide a reference for the quality control of *H. hedyotidea* and the formulation of quality standard for *H. hedyotidea*.

H. hedyotidea is a commonly used traditional Chinese medicine, it is the first drug in the formula of Zhonghua Dieda Pill recorded in the *Chinese Pharmacopoeia*[4], and is also a main drug in the formula of Sanshedan Chuanbei Syrup[5], Bairong Zhike Syrup[6], Shedan Chuanbei Syrup[7], *etc*. Besides, *H. hedyotidea* is a common medicine of Zhuang and Yao people[8-9]. In addition, it has also been used as the main raw material of natural sweet plants in Zhuang people areas. In summary, *H. hedyotidea* can be widely used in pharmaceutical and food and beverage industries, and can be used as a raw material to develop a natural sweetener to replace sugar to be made into beverages, cakes, sherbet, soda, jelly and the like. It can also be used to develop new health food from traditional national food, so it has good application value and market prospects. Therefore, it is of practical significance to study the microscopic characteristics and conventional detection of *H. hedyotidea*.

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In summary, the main active components, pathways and targets of Ningmitai capsule for the treatment of UTIs were predicted and screened. The results showed that there were 37 main active components, including salicin, ferulic acid, wogonin, ellagic acid, vanillic acid, ursolic acid, kaempferol, jatrorrhizine, isoorientin, quercetin, *etc.* In addition, Ningmitai capsule for the treatment of UTIs may mainly act on NFKB1, JUN, CTNNB1, TP53, MYC, CDK1, STAT3, HDAC1, EP300, CTNNB1 and other targets, and play a role in the treatment of UTIs through prostate cancer, pancreatic cancer, chagas disease, bladder cancer, toxoplasmosis and other pathways. These preliminary results provide a basis for further study of pharmacological mechanism and clinical application of Ningmitai capsule.

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