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Original article

Comparative pharmacognosy of *Pyrrosia petiolosa* and *Pyrrosia davidii*

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ABSTRACT

Pyrrosia petiolosa (Christ) Ching, Polypodiaceae, is an important medicinal pteridophyte used for the treatment of nephritis and bronchitis, while *P. davidii* (Giesenhagen. ex Diels) Ching, Polypodiaceae, often substitutes medicinal *Pyrrosia* in clinic. The present study was aimed to compare the pharmacognosy of *P. petiolosa* and *P. davidii*, including plant morphology, microscopic characteristics, physico-chemical parameters, UV and IR spectrum, and HPLC fingerprint. It was revealed that the two herbs had basically similar pharmacognostical characteristics but with certain differences. The present study contributes to the standardization and verification of these medicinal materials.

Introduction

Pyrrosia petiolosa (Christ) Ching, Polypodiaceae, is an important medicinal pteridophyte, which grows in wet places of mountain bare rocks or rock seam in the region of northern China and the middle and lower reaches of Yangtze River (Wang et al., 2006). The dried leaves of *Pyrrosia* are used as medicinal materials to treat gonorrhea. At present, three *Pyrrosia* plants are recorded in the Chinese Pharmacopoeia, including *Pyrrosia petiolosa* (Christ) Ching, *P. sheareri* (Baker) Ching and *P. lingua* (Thunb.) Farw (Chinese Pharmacopoeia, 2010). Several studies have been carried out in recent years (Hsu, 2008; Zhang et al., 2014), to elucidate the chemical constituents and pharmacology of *P. petiolosa* (Jong et al., 2000). However, further studies have shown that the efficacy and chemical constituents of *Pyrrosia* varied between environments and species, resulting in inconsistent clinical effects (Yang et al., 2003; Ma et al., 2006). On the other hand, some non-medicinal *Pyrrosia* species, such as *P. davidii* (Giesenh. ex Diels) Ching, have been used to substitute for medicinal *Pyrrosia*. These substitutes have not been recorded in the Chinese pharmacopoeia, however, many of them have a wide circulation in the market and display good clinical efficacy (Mi et al., 2012). For pharmaceutical workers, it is difficult to distinguish *P. petiolosa* from *P. davidii*, especially from their parts (Shi et al., 2007). Therefore, in the present work, the detailed pharmacognostical studies including morphological and microscopical characteristics, physico-chemical parameters and chemical constituents fingerprints of *P. petiolosa* and *P. davidii*.

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Materials and methods

Materials and reagents

The fresh materials were collected in Mengshan Mountains (N 35° 31′ 30″, E 117° 54′ 46″, Height: 450 m), Jiaodong area (N 36° 48′ 30″, E 121° 18′ 45″, Height: 360 m) in the Shandong province, China. At the Changbai Mountains (N 43° 29′ 98″, E 126° 09′ 76″, Height: 480 m) in the Jilin province, China. Some *Pyrrosia* medicinal materials were purchased from medicine markets of Anhui, Zhejiang and Yunnan, China. The species were identified by Gao Demin, an associate professor of Shandong University of TCM. The voucher specimens (SDCM 201220, SDCM 201221) were deposited in the Herbarium of Shandong University of TCM (SDCM). All chemical reagents were of analytical grade.

Microscopic identification

Temporary slides of leaf blades, petiole, rhizome and powder of *P. petiolosa* and *P. davidii* (twelve samples from each collection) were prepared and observed under light microscope (Kannan et al., 2012; Rose and Prasad, 2013). For the scanning electron microscopy (SEM) analysis of *Pyrrosia* samples were fixed in FAA, dehydrated in a graded ethanol series, then dried, mounted and coated, and photographs were taken using an EVO40 SEM (Carl Zeiss, Germany).

Physical and chemical color reaction and TLC analysis

The powders of *P. petiolosa* and *P. davidii* leaves and rhizomes were extracted with 80% ethanol under reflux for 4 h, twice. The extracts were combined, filtered, and concentrated for color reaction (Alam and Gupta, 1986). The physical and chemical color reactions were performed as previously described (Chadwick et al., 2006).

Flavonoids and anthraquinones were identified using hydrochloric acid-magnesium reaction and alkali reaction, respectively. Triterpenes were yielded using Rosen-Heimer reaction. Polysaccharides were identified using the periodic acid Schiff reaction. Tannins and saponins were analyzed using ferric trichloride, anisaldehyde-sulfuric acid and Lieberman-Burchard reaction respectively (Mandal and Kumar, 2002; Xiang et al., 2002).

Extracts of *P. petiolosa* and *P. davidii* (20 µl) were placed on polyamide-6-layer sheets (Sinopharm Chemical Reagent Co., Ltd), eluted with SDS, C_4H_9OH, C_7H_{16} (27:63:10, v/v/v) microemulsions (containing 75% water), revealed with anisaldehyde-sulfuric acid, and the retention factors (R_f) were determined.

Histochemical localization

Histochemical localizations of polysaccharides, flavonoids, saponins and anthraquinones were performed according to previous reports (Beaumont et al., 1986; Harborne, 1998).

The determination of physico-chemical parameters

The moisture, total ash and acid-insoluble ash, water-soluble extractives and ethanol-soluble extractives were determined according to the methods recorded in the Chinese Pharmacopoeia (2010). The experiments were repeated five times.

The determination of UV-VIS spectrum

P. petiolosa and *P. davidii* extracts were obtained using distilled water, ethanol (70%) and petroleum ether, respectively (Bruni and Tosi, 1982). The obtained samples were treated and further analyzed using UV-VIS absorption spectrophotometry (Kalyuzhny et al., 2000; Liang et al., 2010). The experiments were performed five times. Twelve samples came from previously collected *Pyrrosia* materials.

HPLC fingerprint

Methanol (50%) extracts of *P. petiolosa* and *P. davidii* were prepared (Cai et al., 2012). The HPLC analysis was carried out on Sino Chrom ODS-BP column (4.6 mm×250 mm, 5 µm) using a mobile phase of acetonitrile and phosphoric acid 0.5% solution (gradient elution: 0-10 min, 8%-9%; 10-50 min, 9%-13%) at a flow rate of 1.0 ml/min^{-1}, and a detection wavelength of 326 nm (Zhang et al., 2011). The experiments were repeated five times and twelve samples were obtained from collected *Pyrrosia* materials.

The determination of chlorogenic acid

The aqueous, methanol (50%), ethanol (70%), acetone and *n*-butanol, ethyl acetate and petroleum ether extracts of roots and leaves were filtered through a 45 µm mesh filter, and used for the determination of chlorogenic acid by HPLC (Zhang et al., 2012). The HPLC analysis was performed as above-mentioned.

IR spectrum

The IR spectra of methanol, acetone extracts, leaves and roots powder of *P. petiolosa* and *P. davidii* were performed as previously described (Heneczkowski et al., 2001). The experiments were carried out for six samples from different areas.

Results

Morphological characteristics

Pyrrosia petiolosa and *P. davidii* are perennial evergreen plants. Their leaves show significant differences in shape, size, thickness and texture.

Pyrrosia petiolosa

Presents a long creeping rhizome, 1-2.5 mm in diameter. Its phyllopodia are 0.5-2.5 cm apart, with a few scattered sclerenchyma strands obvious in a cross section. The hydathodes are distinct sunken and the indument is persistent, monomorphic, dense, and colored light gray to brown with appressed, boat-shaped rays and hairs that are 0.4-0.6 mm in diam. The sori are superficial, without a central bundle of paraphyses (Fig. 1, Chart 1).

Chart 1
Parts of morphological and microscopic characteristics of *Pyrrosia petiolosa* and *Pyrrosia davidii*.

N°	Parameters		*Pyrrosia petiolosa*	*Pyrrosia davidii*
1	Macroscopic characters	plant height (cm)	4-17	5-25
2		leaf types	dimorphic, trophophyll and sporophyll, ovate	monomorphic, sporophyll, lanceolate
3		petiole length (cm)	2-3 (trophophyll), 6-12 (sporophyll)	2-5
4		leaf blade length (cm)	3-4 (trophophyll), 4-6 (sporophyll)	5-15
5		leaf blade width (mm)	10-18 (trophophyll), 10-20 (sporophyll)	8-14
6		colour	grey brown (leaf) light brown (scales)	pale green (leaf) black brown (scales)
7	Stellate hair	overall size	larger	smaller
8		branches numbers	6-11	5-10
9		branches width (μm)	50-70	10-48
10		branches length (μm)	260-380	200-570
11		handle cells	3-9	3-5
12		handle length (μm)	180-610	150-280
13	Transverse section of blade	the inside of the principal veins under the skin	a small amount of sclerenchyma	no sclerenchyma
14	Transverse section of petiole	monostele	6-9	6-10
15		xylem type	'V'	'T'

All microscopic characteristics were studied using a biomicroscope (10×40).

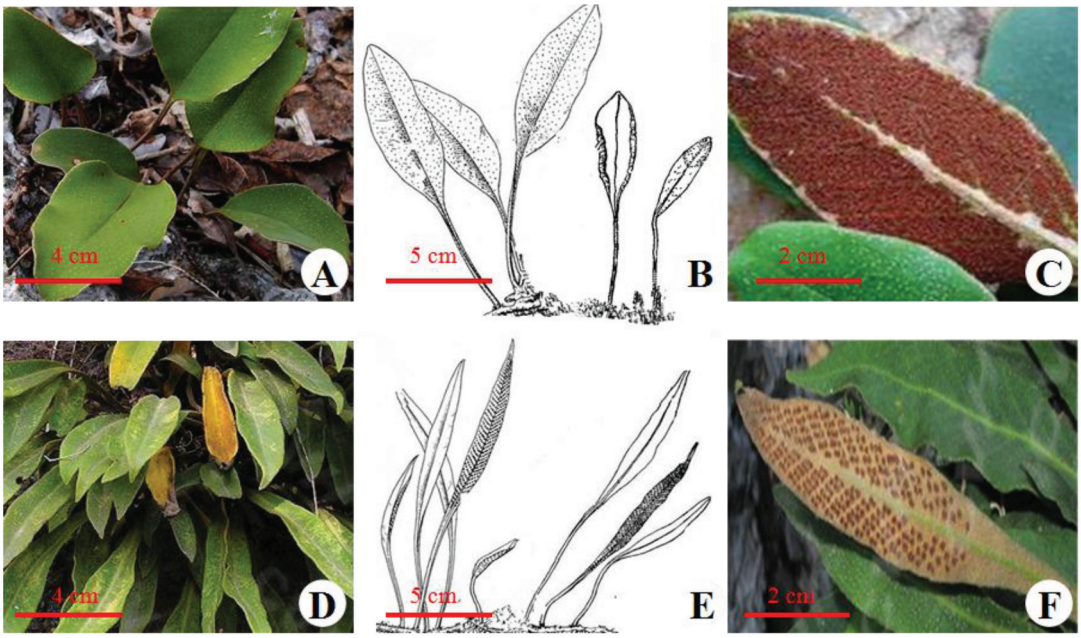

Figure 1 – Macroscopic characteristics of *Pyrrosia petiolosa* and *Pyrrosia davidii*. A. Macroscopic characteristics of *Pyrrosia petiolosa*; B. Macroscopic characteristics of *Pyrrosia petiolosa*; C. Macroscopic characteristics of sporophyll of *Pyrrosia petiolosa*; D. Macroscopic characteristics of *Pyrrosia davidii*; E. Macroscopic characteristics of *Pyrrosia davidii*; F. Macroscopic characteristics of sporophyll of *Pyrrosia davidii*.

Pyrrosia davidii

Presents a short elongated rhizome, 1.6-3.1 mm in diam; the cross-section displays few too many sclerenchyma strands; phyllopodia 0.3-0.7 cm apart. The fronds are monomorphic, with gradually narrowed bases and an acute to acuminate apex. The sori are superficial, without central bundle of paraphyses. The sporangia are contained in long stalks and embedded in capsules (Fig. 1, Chart 1).

Microscopical characteristics

Transverse section of the leaf blade and petiole

The transverse section of the leaf blade and petiole were very similar. The external periclinal cell walls of the upper epidermis cell were wavy. The epidermis was uniseriate and cuticle covered. A 3-4 layered sclerenchymatous hypodermis was encountered above collenchymatous ground tissue. Palisade tissue was composed of 2-3 layers of closely packed cells and spongy tissue composed of 5-8 columns of closely packed irregular parenchyma. The vessel elements in *P. petiolosa* were arranged in a 'V' shape, while those in *P. davidii* were arranged in a 'T' shape. A mass of sclerenchyma was found beneath the vascular cylinder in the transverse sections of *P. davidii* leaf blade and petiole. Phloem surrounds the xylem in both species (Figs. 2 and 3, Chart 1).

Leaf blade epidermis

The SEM showed that epidermal cells in *P. petiolosa* were elongated, while those in *P. davidii* were round or irregularly shaped. Stomata were only present on the abaxial side of the leaf blades in both species, but the distribution pattern differed, with 10-16 per mm^2 in *P. petiolosa* and 15-20 per mm^2 in *P. davidii* (Fig. 3).

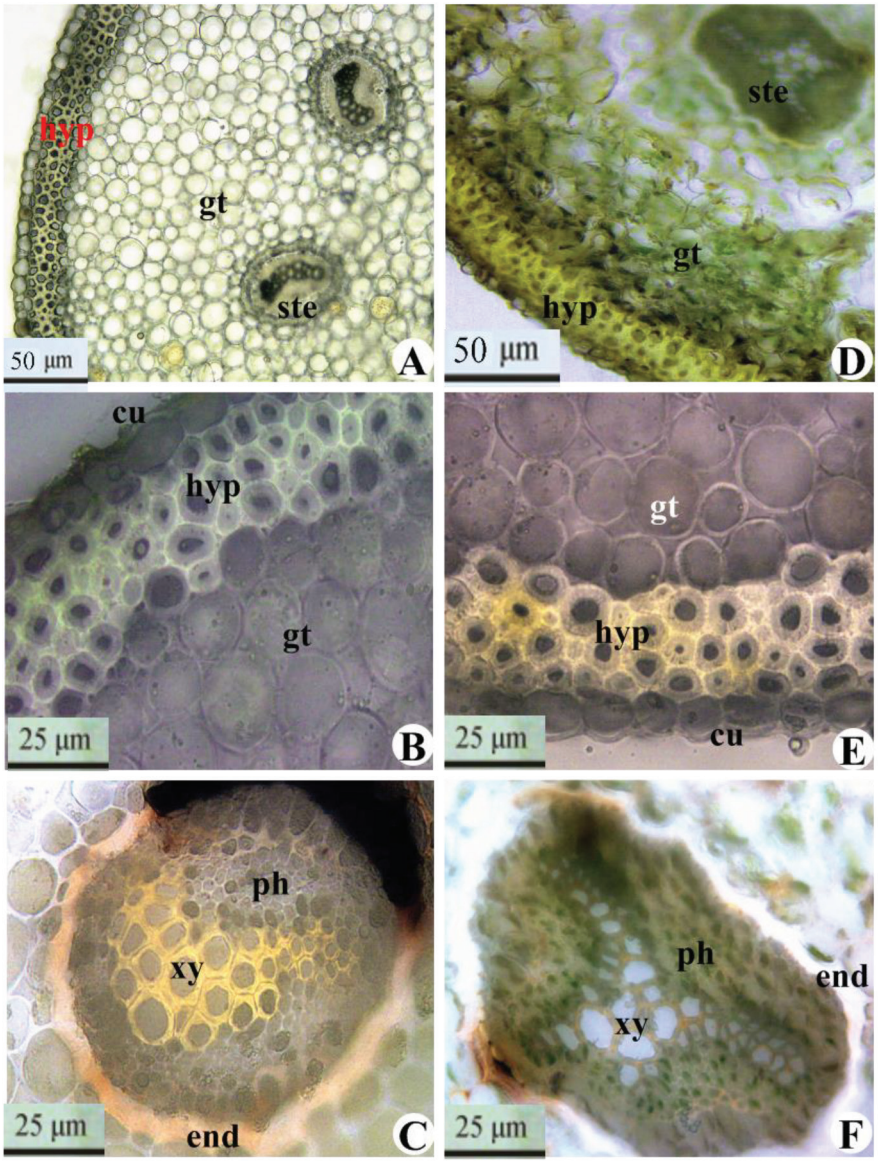

Figure 2 – Characteristics microstructures of *Pyrrosia petiolosa* and *Pyrrosia davidii* petiole in transverse section. A, B and C. *Pyrrosia petiolosa*; D, E and F. *Pyrrosia davidii*. Cu, cuticle; end, endodermis; gt, ground tissue; hyp, hypodermis; ph, phloem; ste, stelexy; xy: xylem.

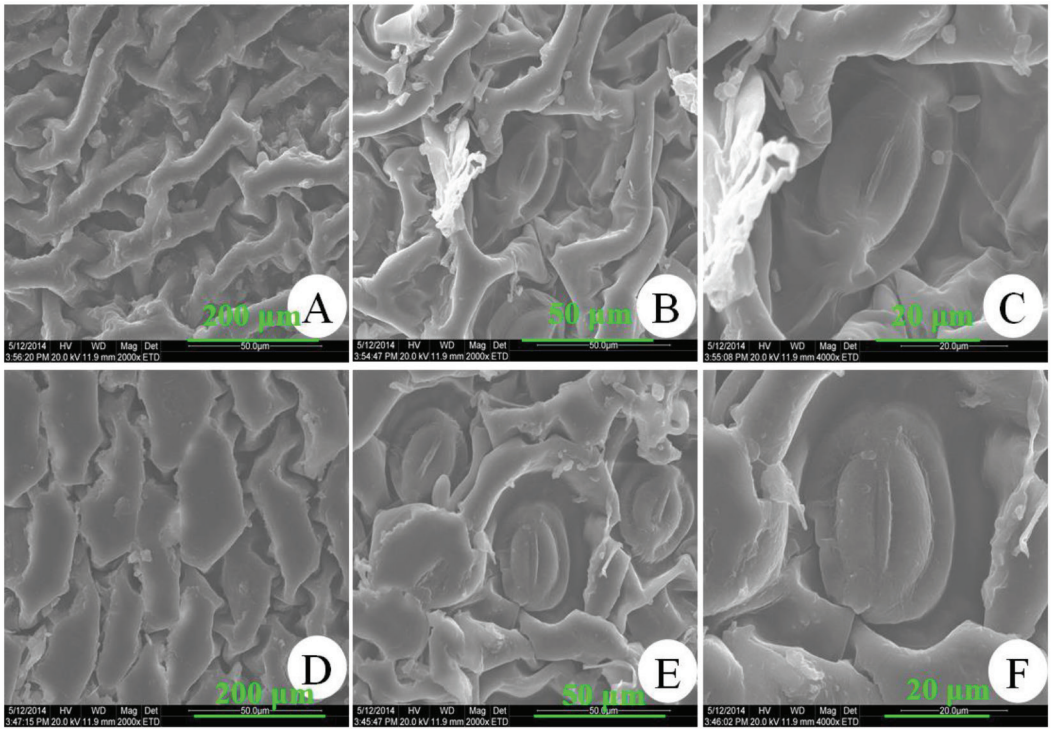

Figure 3 – Microstructure of *Pyrrosia petiolosa* and *Pyrrosia davidii* leaf blade epidermis. A. Adaxial surface of the leaf in *Pyrrosia petiolosa*; B. Abaxial surface of the leaf in *Pyrrosia petiolosa*; C. Stomata of *Pyrrosia petiolosa*; D. Adaxial surface of the leaf in *Pyrrosia davidii*; E. Abaxial surface of the leaf in *Pyrrosia davidii*; F. Stomata of *Pyrrosia davidii*.

Fronds powder

As are shown in Figs. 4, 5 and 6, powder from the fronds of both species had a large amount of stellate hairs, fibers, stone cells, vessels, spores, sporangia filled with spores, and sporangium bands, but significant differences were found in the morphological characteristics of sporangium bands, spores and stellate hairs. The spores in *P. petiolosa* were ovoid with a sparse warty surface, while those in *P. davidii* were kidney-shaped with a dense warty surface (Figs. 4, 5 and 6).

Transverse section of the rhizomes

The structures of the transverse sections of rhizomes were very similar. There were specialized epidermis and scales in two columns of the epidermal cells; 2-5 columns of fiber cells were arranged in a circle inside of the epidermal cells. The amphicribral vascular bundle was composed of 6-11 vascular cylinders. Secretory cells exist in the parenchyma.

Physical and chemical color reactions and TLC

Physical and chemical color reactions indicated the presence of flavonoids, tannins, saponins, anthraquinones, triterpenes and polysaccharides. In addition, the contents of flavonoids, saponins and anthraquinones of *P. petiolosa* were higher than those in *P. davidii*. TLC analysis showed significant differences between *P. petiolosa* and *P. davidii*. Judged by the brightness of spots, the content of chlorogenic acid in *P. petiolosa* was significantly higher than that in *P. davidii* (Fig. 7).

Histochemical localization

P. petiolosa and *P. davidii* contain similar chemical constituents, including flavonoids, anthraquinones, triterpenes, steroids and tannins, but concentrations were significantly different. The contents of flavonoids, saponins and anthraquinone in *P. petiolosa* were higher than those in *P. davidii* (Table 1).

Physico-chemical parameters

Moisture and total ash content of *P. petiolosa* were similar to those of *P. davidii*. However, the contents of acid-insoluble ash, aqueous, ethanol and petroleum ether extracts between *P. petiolosa* and *P. davidii* showed significant differences. All parameter values of *P. petiolosa* were higher than those of *P. davidii* except in the petroleum ether-soluble extract (Table 2).

UV-VIS spectrum

The UV-VIS spectra and their second derivatives of alcohol and ether extract were similar, while the aqueous extract was different. Therefore, the second derivative UV-VIS spectra of the aqueous extract can be used to distinguish *P. petiolosa* from *P. davidii*. Also, intensity peaks at 220, 242, 263, 302, 249 (from *P. davidii*) and 251, 299, 325 (from *P. petiolosa*) were significantly different (Fig. 8).

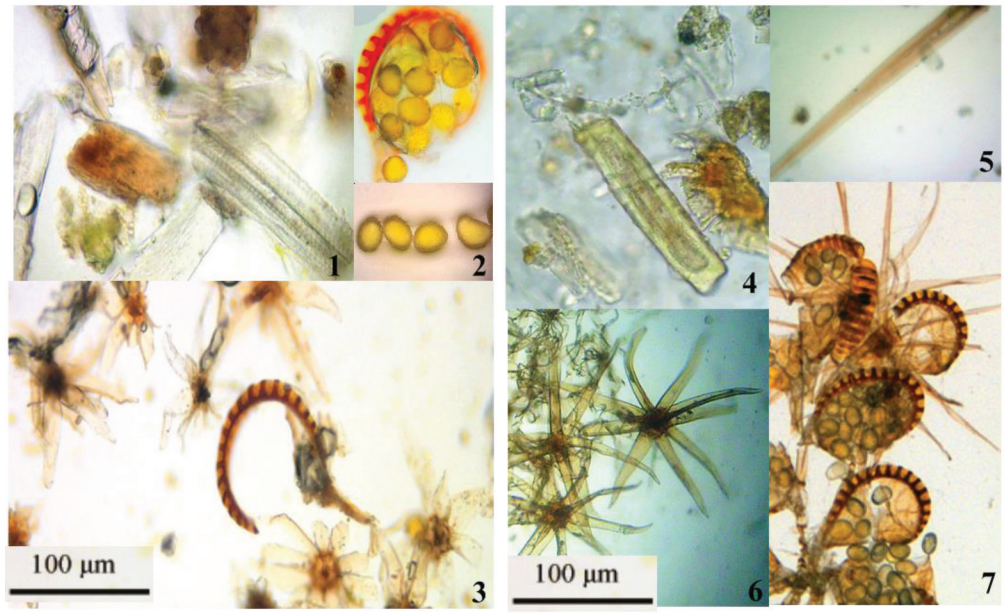

Figure 4 – Microstructure characteristics of *Pyrrosia petiolosa* and *Pyrrosia davidii* fronds powder. 1. Trachea of *Pyrrosia petiolosa*; 2. Spores and sporangium band of *Pyrrosia petiolosa*; 3. Stellate hairs of *Pyrrosia petiolosa*; 4. Stone cells of *Pyrrosia davidii*; 5. Fiber of *Pyrrosia davidii*; 6. Stellate hairs of *Pyrrosia davidii*; 7. Spores and sporangium band of *Pyrrosia davidii*.

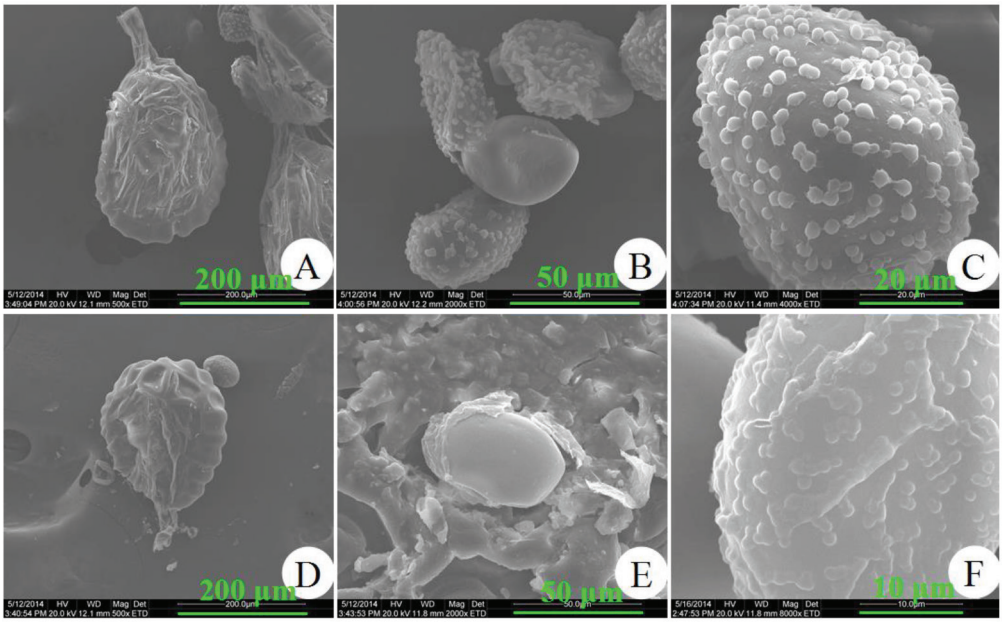

Figure 5 – Microscopic characteristics of *Pyrrosia petiolosa* and *Pyrrosia davidii* sporangia and spore. A, B and C. *Pyrrosia petiolosa*; D, E and F. *Pyrrosia davidii*.

HPLC fingerprint

HPLC fingerprints of *P. petiolosa* and *P. davidii* were similar; however, the contents of each ingredient from the peak position in *P. petiolosa* and *P. davidii* were different, especially at the retention time of the 11th min (Fig. 9, Table 3).

Chlorogenic acid

The chlorogenic acid mainly is present in the ethanol, methanol, methanol 50% and aqueous extract of leaves. The chlorogenic acid content in the different extracts of *P. petiolosa* and *P. davidii* were significantly different and the content was higher in *P. petiolosa* than that in *P. davidii* (Table 4).

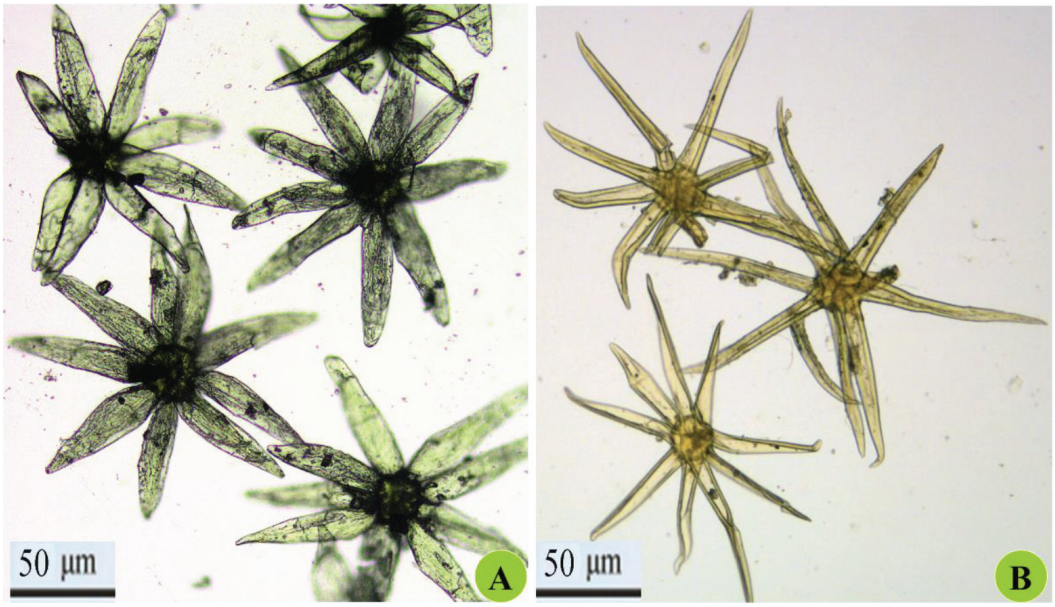

Figure 6 – Microscopic characteristics of *Pyrrosia petiolosa* and *Pyrrosia davidii* stellate hairs. A. *Pyrrosia petiolosa* stellate hairs; B. *Pyrrosia davidii* stellate hairs.

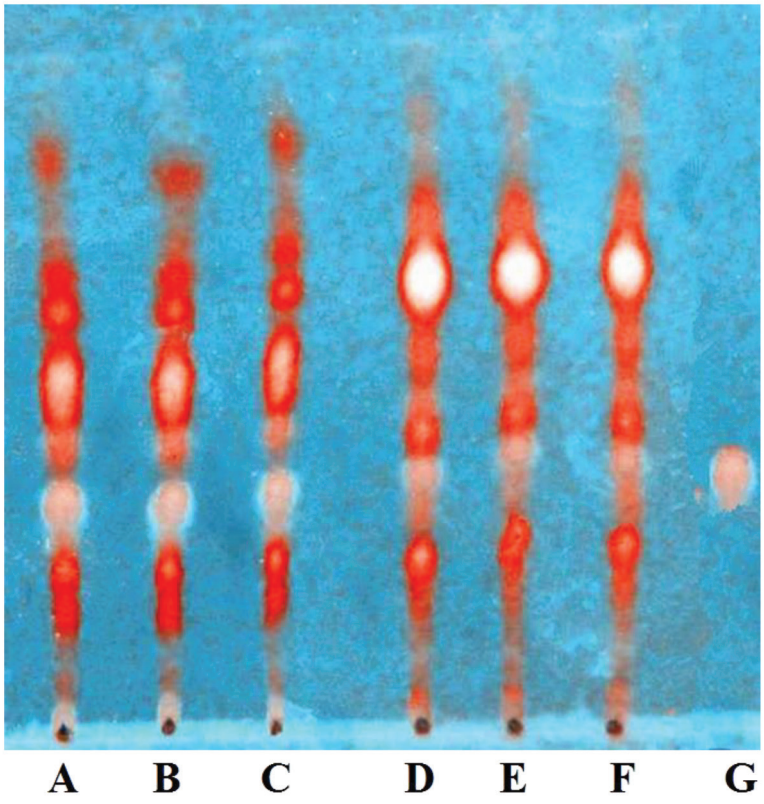

Figure 7 – TLC of *Pyrrosia petiolosa*, *Pyrrosia davidii* and Chlorogenic acid. A, B and C. *Pyrrosia petiolosa* (three samples from different areas); D, E and F. *Pyrrosia davidii* (three samples from different areas); G. Chlorogenic acid.

Table 1
Parts of morphological and microscopic characteristics of *Pyrrosia petiolosa* and *Pyrrosia davidii*.

Herbs	N°	Location	Polysaccharides	Flavonoids	Saponins	Anthraquinone
Pyrrosia petiolosa	1	Epidermis	+	+++	+++	++
	2	Palisade tissue	+	++	+++	+++
	3	Spongy tissue	+	++	++	++
	4	Collenchyma	++	+++	++	–
	5	Phloem	+	++	++	++
	6	Xylem	+	–	–	–
Pyrrosia davidii	7	Epidermis	+	++	+	+
	8	Palisade tissue	+	+	++	++
	9	Spongy tissue	+	+	+	+
	10	Collenchyma	++	++	+	–
	11	Phloem	+	+	+	+
	12	Xylem	+	–	–	–

+, present; –, absent; n = 10 per sample. The number of (+) is representative of the degree.

Table 2
Phytochemical parameters of *Pyrrosia petiolosa* and *Pyrrosia davidii*.

N°	Parameters	*Pyrrosia petiolosa* (%)	*Pyrrosia davidii* (%)
1	Moisture content	8.90 ± 0.078	6.74 ± 0.102
2	Ash content	4.99 ± 0.142	4.62 ± 0.082
3	Acid insoluble ash content	0.53 ± 0.033	1.29 ± 0.031
4	Water-soluble extract content	18.18 ± 0.172	25.49 ± 0.480
5	Alcohol soluble extract content	15.92 ± 0.262	20.64 ± 0.445
6	Ether soluble extract content	9.68 ± 0.872	6.73 ± 0.266

Values are mean % ± SD. The values were from three independent replicates.

Table 3
The compound content of different peaks well defined from *Pyrrosia petiolosa* and *Pyrrosia davidii*.

N°	*Pyrrosia petiolosa* (%)	*Pyrrosia davidii* (%)	N°	*Pyrrosia petiolosa* (%)	*Pyrrosia davidii* (%)
1	60.80 ± 3.80	43.70 ± 2.80	8	–	2.34 ± 0.32
2	8.52 ± 0.86	6.84 ± 0.76	9	1.16 ± 0.12	3.01 ± 0.32
3	7.25 ± 0.56	5.65 ± 0.56	10	6.15 ± 0.57	2.60 ± 0.37
4	1.19 ± 0.20	0.42 ± 0.30	11	–	6.87 ± 0.69
5	1.55 ± 0.16	0.97 ± 0.16	12	0.45 ± 0.06	9.12 ± 0.86
6	0.54 ± 0.04	0.80 ± 0.07	13	0.61 ± 0.03	0.80 ± 0.03
7	1.43 ± 0.09	0.61 ± 0.09			

The values were expressed as mean ± SD of five independent replicates.

Table 4
The content of chlorogenic acid in different extracts of *Pyrrosia petiolosa* and *Pyrrosia davidii*.

Extract	*Pyrrosia petiolosa* (%)		*Pyrrosia davidii* (%)	
	Leaves	Roots	Leaves	Roots
Aqueous	5.44 ± 0.125	1.07 ± 0.091	2.17 ± 0.082	**0.41 ± 0.023**
Methanol (50%)	6.90 ± 0.232	0.94 ± 0.038	3.63 ± 0.125	1.04 ± 0.061
Methanol	7.10 ± 0.252	1.27 ± 0.045	4.40 ± 0.116	1.07 ± 0.052
Ethanol (70%)	8.13 ± 0.173	1.60 ± 0.036	5.53 ± 0.093	**1.17 ± 0.075**
Acetone	0.78 ± 0.009	0.60 ± 0.007	0.62 ± 0.082	0.39 ± 0.021
n-butanol	0.70 ± 0.005	0.13 ± 0.008	0.37 ± 0.035	0.07 ± 0.003
Ethyl acetate	0.44 ± 0.008	0.09 ± 0.005	0.41 ± 0.046	0.16 ± 0.0007
Petroleum ether	0.02 ± 0.001	0.01 ± 0.0009	–	–

The values were expressed as mean ± SD of five independent replicates.

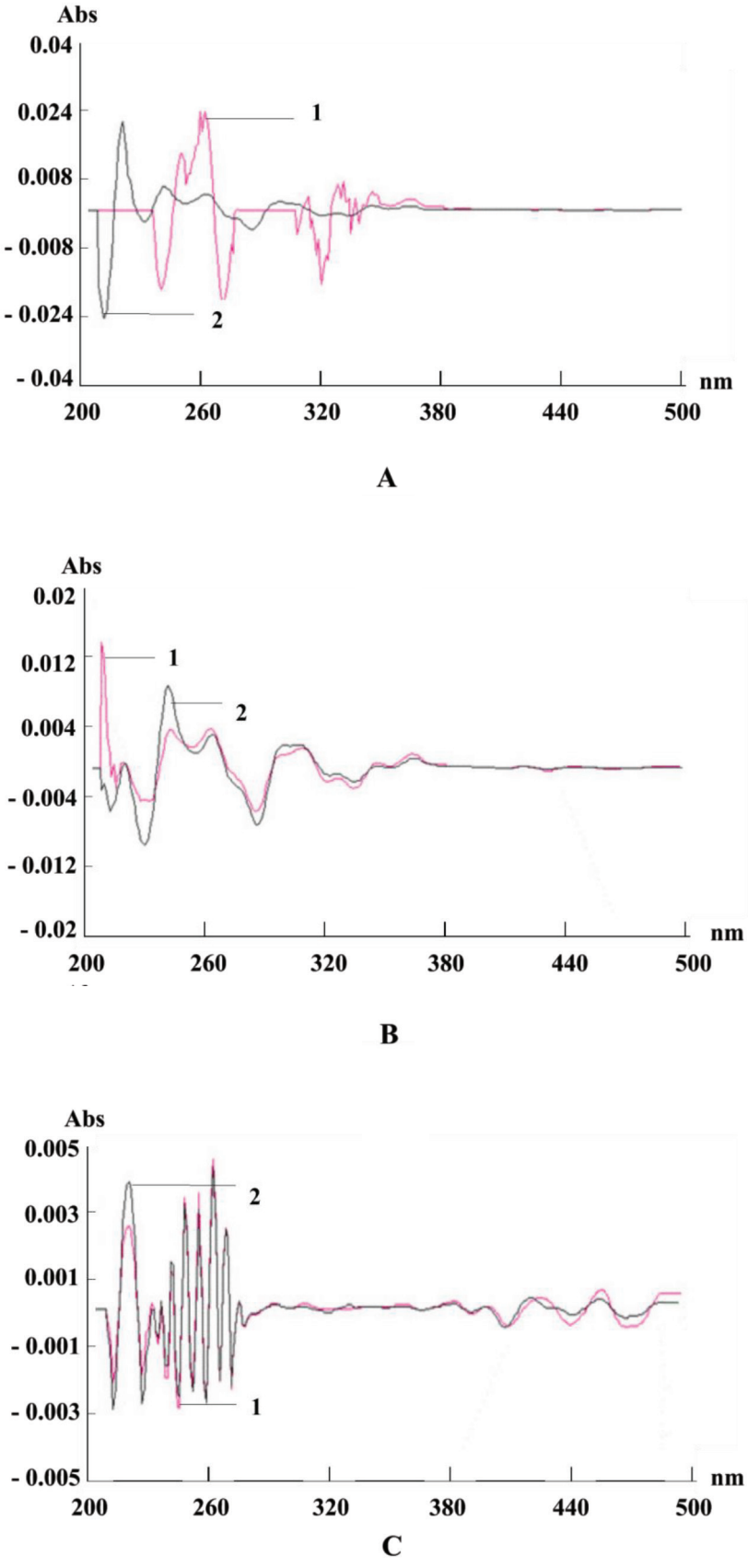

Figure 8 – The second derivative of ultraviolet-visible spectra of *Pyrrosia petiolosa* and *Pyrrosia davidii*. 1. *Pyrrosia petiolosa*; 2. *Pyrrosia davidii*. A. Aqueous soluble extract; B. Ethanol soluble extract; C. Petroleum ether soluble extract.

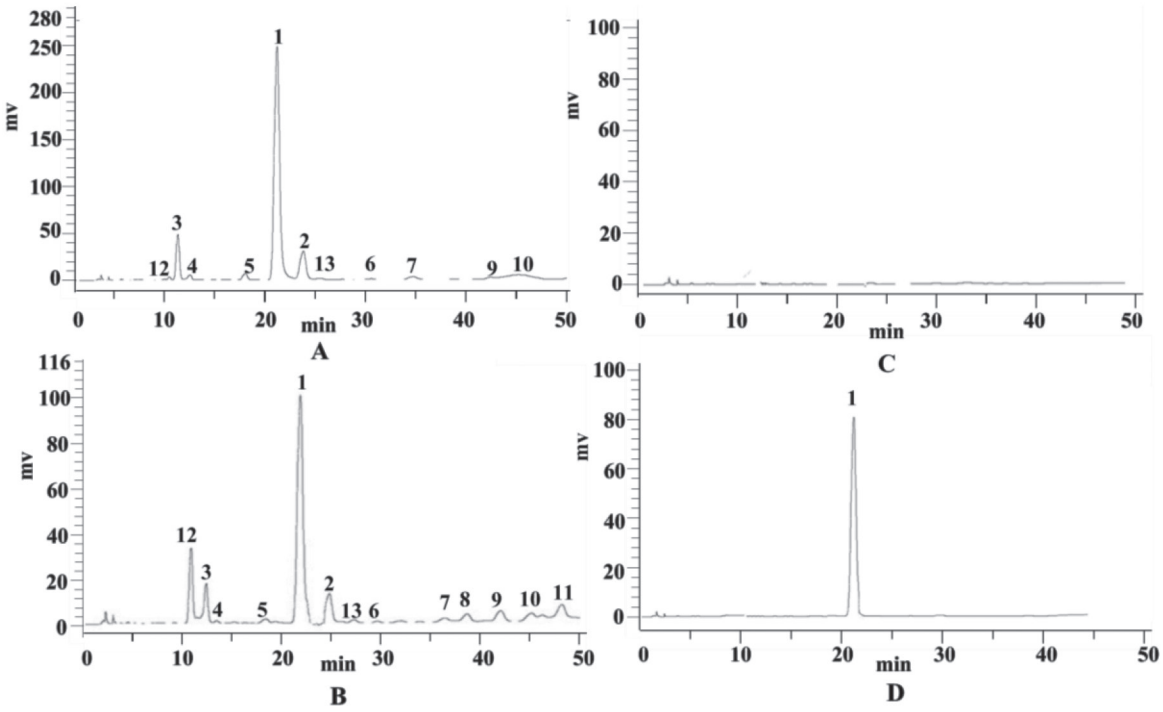

Figure 9 – The HPLC fingerprints of *Pyrrosia petiolosa* and *Pyrrosia davidii*. A. *Pyrrosia petiolosa*; B. *Pyrrosia davidii*; C. Negative control; D. Chlorogenic acid. Wavelength, 326 nm; Mobile phase, acetonitrile (A) 0.5% phosphoric acid solution; (B) 0-10 min, 8-9% (A); 10-50 min, 9%-13% (A); Flow rate, 1.0 ml.min^{-1}. HPLC fingerprints were representative from five experiments and six specimens.

IR spectrum

P. petiolosa and *P. davidii* contain consistent functional groups (4000-1300 cm^{-1}), such as alcohol hydroxyl, phenolic hydroxyl and carboxyl, fat hydroxyl, carbonyl, and benzene. However, FTIR spectra fingerprint region (1300-400 cm^{-1}) had significant differences.

In *P. petiolosa*, carbohydrates such as glycogen were shown in the spectral output at 1249, 1050 cm^{-1} in the leaves and 1240, 1036 cm^{-1} in the roots; while in *P. davidii*, spectral output at 1253, 1060 cm^{-1} in the leaves and 1247, 1034 cm^{-1} in the roots. These were ascribed to stretching vibrations of hydrogen-bonded C-O groups; esters participated in the spectral output at 1738, 1249 cm^{-1} in *P. petiolosa* leaves, 1738, 1253 cm^{-1} in *P. davidii* leaves. These were associated to stretching vibrations of hydrogen-bonded C=O, C-O groups. Amides of the spectral output had peaks at 3391, 1639 cm^{-1} in *P. petiolosa* leaves, and 3376, 1640 cm^{-1} in *P. davidii* leaves.

The principal differences were the presence of bands at 1544 cm^{-1}, possibly assigned to an unsaturated fat structure, and at 3605 cm^{-1} associated with an asymmetric O-H, N-H stretching of *P. petiolosa*, but neither appeared in *P. davidii*.

The FTIR spectra fingerprint region (1300-400 cm^{-1}) of *P. petiolosa* and *P. davidii* showed pronounced differences. They displayed only one common peak at 469 cm^{-1}. The peak at 470, 414 cm^{-1} only appeared in *P. petiolosa* leaves, while the peak at 815, 766 cm^{-1} only appeared in *P. davidii* leaves. The peaks at 648, 434 cm^{-1} only appeared in *P. petiolosa* roots (Fig. 10, Table 5).

Discussion

The most noticeable differences between *P. petiolosa* and *P. davidii* were the morphological and microscopical characteristics, important factors to guarantee the quality of medicinal materials and clinical efficacy.

The medicinal materials displayed differences in size, shape, texture, and thickness of the leaves, rhizomes, serrated edges, and hair roots, as well as the types of leaves and the size of the petiole. Regarding microscopic characteristics, the wide and short stellate hair branches, long stellate hairs handle and its 'V' xylem of *P. petiolosa* were distinguished from those of *P. davidii*.

The types, content, and distribution of effective components can be concluded from physical and chemical reactions and histochemical localization, which provided the basis for the quality analysis of the medicinal materials for clinical use. *P. petiolosa* and *P. davidii* contain the same chemical compositions, including flavonoids, anthraquinones, carbohydrates, triterpenes, steroids, tannins. Flavonoids, anthraquinones and saponins were mainly distributed in the palisade tissue of the leaf, while the concentration of anthraquinones in the xylem and epidermis was not obvious (Khatoon et al., 2006). The content of anthraquinones was higher than saponins in the collenchyma. In *P. petiolosa*, polysaccharides were mainly distributed in the mesophyll and the vascular bundle parenchyma, and a small amount was located in the epidermis. TLC fingerprint profiles of *P. petiolosa* and P. davidi showed the presence of eight spots, of which the spot at R_f 0.28, viz., chlorogenic acid can be used to easily differentiate *P. petiolosa* and *P. davidii*.

Table 5
Distribution of FTIR spectra of *Pyrrosia petiolosa* and *Pyrrosia davidii* leaves, roots and acetone extraction (cm^{-1}).

	Leaves		Roots		Acetone extraction	
N°	PP	PD	PP	PD	PP	PD
1	3391	3376	3334	3329	3456	–
2	2921	2921	2924	2925	3016	3017
3	2853	2854	2851	2852	2970, –	2970, 2947
4	1738	1738	–	–	1739, –	1739, 1729
5	1639	1640	1644	1644	–	–
6	1520	1520	1542	1541	1435	1435
7	1436	1442	1438	1440	1455	1455
8	1371	1379	1374	1376	1355, 1365, 1369	1355, 1360, 1365
9	1249	1253	1240	1247	1229, 1217, 1206	1229, 1217, 1206
10	1050	1060	1036	1034	1092	1092
11	–	815	778	779	896	896
12	–	766	694	696	–	780
13	601	558	648	–	538	540
14	470	–	533	518	515	516
15	444	442	469	469	527	527
16	414	–	434	–	426	427
17	407	408	402	403	403	403

The values were expressed as mean ± SD of five independent replicates.

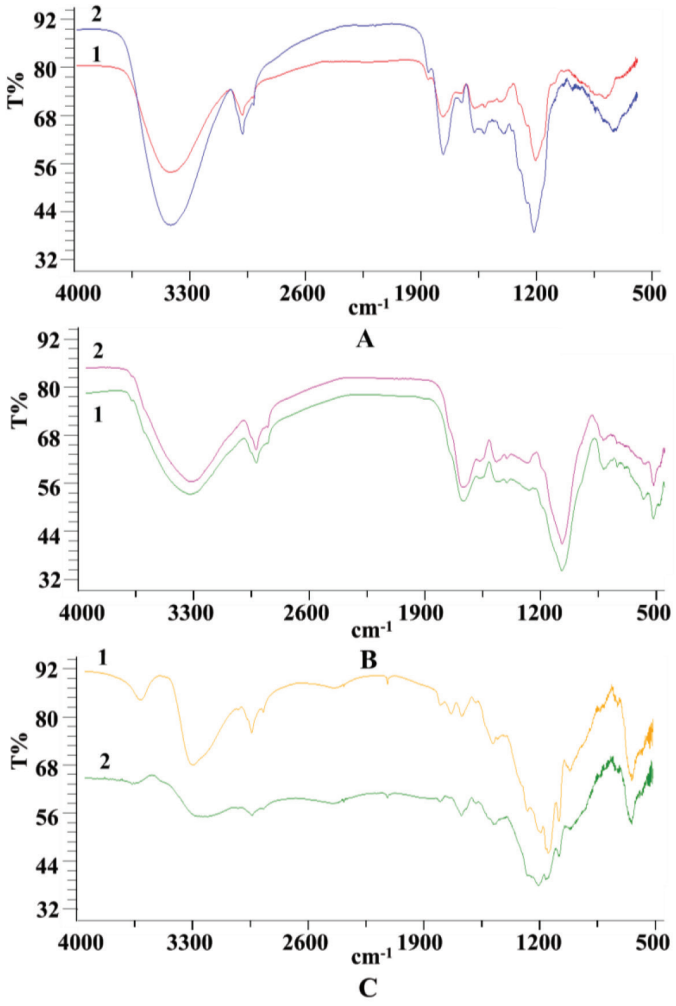

Figure 10 – The comparison of FTIR spectra of *Pyrrosia petiolosa* and *Pyrrosia davidii* leaves, roots, methanol extraction. A. leaves; B. roots; C. methanol extract; 1. *Pyrrosia petiolosa*; 2. *Pyrrosia davidii*.

Chlorogenic acid is not only an active component, but also an important reference component (Johnston et al., 2003). The content of chlorogenic acid in *Pyrrosia* leaf was no less than 2% by HPLC method (Chinese Pharmacopoeia, 2010). Although the content of chlorogenic acid in *P. petiolosa* and *P. davidii* both reached the standard as medical materials, the amount in the former was significantly higher than that in the latter. This partly explains the fact that *P. petiolosa* is more widely used in the clinic and circulated in the market.

It has been reported that mangiferin and polyphenols were important active components (Garcia et al., 2003; Sugiyama et al., 2007). The content of flavonoids and polyphenols were higher in *P. petiolosa* than that in *P. davidii* by analyzing histochemical locations, UV-VIS spectra and HPLC spectrum, which not only provided a quick and easy method for identification of *Pyrrosia* materials, but also workes as a standard HPLC and UV-VIS spectra.

In conclusion, the comparative pharmacognosy analysis of *P. petiolosa* and *P. davidii* provided a base and standard to quickly identify the two plants, which could ensure the safety of natural medicines for clinical use and further promote the development of *Pyrrosia* species.

Authors' contributions

CD carried out most of the experimental work and drafted the manuscript. XX and ZY performed HPLC and UV analysis. GD designed the study, supervised the whole experimental process and edited the manuscript. All the authors have read and approved the final manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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