Pharmacognostic Studies of *Psychotria rubra*(Lour.)Poir

Gengqiu Tang, Xiaojing Lin, Xiudi Lai, Xue Gong, Shengguo Ji

**ABSTRACT**

**Objective:** Various traditional systems of medicine enlightened the importance of *Psychotria rubra* (Lour.) Poir to have a great medicinal value. The present study was carried out to provide a scientific basis of the identification and the authenticity of *Psychotria rubra* (Lour.) Poir with the help of pharmacognostic parameters, which is not done before. **Methods:** Roots, stems, and leaves of *P. rubra* were collected for Pharmacognostic studies involving macros, microscopic evaluation, histochemistry and physicochemical parameters analysis like Ash value, Moisture content, Fluorescence analysis and Thin Layer Chromatography. **Results:** Transverse section of root was found like stone cells, calcium oxalate crystal and vessels in circular shape. Transverse section of stem showed the presence of lenticels and stone cell bands. Transverse section of leaf vein revealed the presence of shaft type of porosity. Powder microscopy revealed the presence of cork cells, stone cells, crystals and resin, fibers, needle crystal beam, thread tracheid and scalariform tracheid. Phytochemical investigation was found like alkaloids, flavones, carbohydrates, saponins, tannins and volatile oils. Results obtained in physicochemical parameters like Total ash value, acid insoluble ash and moisture content were 9.77%, 0.68% and 1.28%, respectively. The leaching degree of *P. rubra* was higher in ethanol(75%, v/v) and methanol solutions than that in chloroform, petroleum ether, and tetrachloromethane solutions. Thin Layer Chromatography (TLC) of the extract revealed 4 spots with the Rf values 0.47, 0.46, 0.46, 0.47 with the solvent system of chloroform: methanol: ethanol(75%, v/v) and methanol solutions than that in chloroform, petroleum ether, and tetrachloromethane solutions. Thin Layer Chromatography (TLC) of the extract revealed 4 spots with the Rf values 0.47, 0.46, 0.46, 0.47 with the solvent system of chloroform: methanol: water: formic acid(7:3:1:0.1). **Conclusion:** The observations confirmed that *P. rubra* has an obvious Pharmacognostic characteristics, which will be useful towards providing a reliable basis for identification, purity, quality and classification of the plant.

**Key words:** *Psychotria rubra*(Lour.), Poir, Identification, Pharmacognostic, Histochemistry, Physicochemical.

**INTRODUCTION**

*Psychotria rubra*(Lour.) Poir, a medicinal plant locally known as “shandayan”, belonging to the genus Psychotria L., family Rubiaceae, is a small evergreen shrub with a height of 0.5-5 meters tall, which widely distributes in tropical and subtropical regions of Asia such as Ryukyu Islands, Taiwan, South China, and Indochina. Normally it grows up in warm and wet environment where the altitude lies from sea level to 20-1500 meters. The leaves are elliptic-oblong, lanceolate-oblong, or rarely oblong-ovate in shape, measuring 5-23.5 cm long and 2-9 cm wide, apex acuminate to acute, base acute to obtuse, margins flat to narrowly revolute, petiole ranges the length from 0.7-5 cm long. Inflorescences are cymose to paniculiform. There are also many distributions on the forest floor in southwest and east China. *P. rubra* has been highlighted the use of its roots, stems and leaves as ‘Traditional Chinese Medicine for the treatment of numerous ailments like cold, diphtheria, dysentery, injury and nameless pain. In addition, the extracts of *P. rubra* have been confirmed having anti-oxidation or anti-inflammation effect and preventing and treating obesity-related diseases. The chemical composition and efficacy of extracts from *P. rubra* were intensively investigated in previous studies. Many new chemical compositions from the volatile oil and ethanol extractive fractions of this plant have been isolated and their structures elucidated. As for its Pharmacological activities, it was reported that different extractive fractions of *P. rubra* showed different efficacy such as anti -depression (ethanolic extract) or improvement on learning and memory (ethyl acetate extract). However, the information regarding its identification of the authenticity is very scanty and poor understood for such studies have not been carried out. As the drug is endowed with huge exploitation and utilization value, it is medicinally important to know precisely and comprehensively about its characteristics of pharmacognosy. In view of importance of knowing about the genuineness of this plant, herein we made a detailed investigation on macroscopic, microscopic characters, histochemistry, physicochemical parameters and fluorescence analysis, and powder
analysis of its roots, stems and leaves to help in identification and standardization of this drug.

**MATERIALS AND METHODS**

**Collection and identification of plant material**

Fresh plants of *P. rubra* were collected from Guangzhou University of Traditional Chinese Medicine, Guangzhou Higher Education Mega Center, taxonomically identified and authenticated by Prof. Shengguo Ji, School of Traditional Chinese Medicine, Guangdong Pharmaceutical University. They were washed and cleaned by flowing water to remove the physical impurities, air-dried under shade, made into a coarse powder using a mechanical blender and preserved in hermetic container with dry air for pharmacognostic study.

**Preparation of sample**

The roots, stems and leaves of *P. rubra* were used for free hand transverse sections, which were immobilized in FAA solution (formalin: glacial acetic acid: 70% ethyl alcohol; [5:5:90]) for macro- and microscopic observations.

**Macroscopic characters**

The organoleptic and macro morphological characters of fresh material including color, shape, size, texture and fracture were studied and noted.

**Microscopic characters**

Fresh material fixed with the FAA was subjected to histologic study. Sliced by paraffin section method, the thin hand cut sections of roots, stems and leaves were dehydrated in a series alcohol concentration, followed by staining with safranine-fast green and mounting with neutral resin. The powders of the drugs were separately treated with glycerine (50%, v/v) and chloral hydrates (10%, v/v) for microscopic study. Microphotographs were taken by observing the free hand sections under Motic Multi-plexer attached to the microscope. All important features were detected and recorded suitably.

**Histochemistry and physicochemical parameters analysis**

The physicochemical parameters analysis of the powders, including ash content (total ash and acid insoluble ash), moisture content, fluorescence analysis, leaching degree and Thin Layer Chromatography, were determined as per the standard guidelines.

**Total Ash value**

The powdered sample of *P. rubra* about 3 g was weighed in a tared silica crucible to incinerate until free from carbon. Gradually increased the temperature to 500-600°C until constant value was obtained.

\[
\% \text{ of total ash value} = \frac{\text{Weight of total ash}}{\text{Weight of sample}} \times 100%
\]

**Acid insoluble ash**

Dilute hydrochloric acid about 10 mL was measured and added to the total ash obtained above in a silica crucible, which was covered with watch glass with water bath heating for 10 minutes. Filtered and residue was washed until the lotion does not show an oxide reaction, dried and burn until constant value was obtained.

\[
\% \text{ of acid insoluble ash value} = \frac{\text{Weight of acid insoluble ash}}{\text{Weight of sample}} \times 100%
\]

**Moisture content**

Moisture content of powders was determined by weighing 2-5 g of powder sample in a weighing bottle dried to constant weight and then placed the weighing bottle in hot air oven at 105°C for 1 hour until constant weight of sample was obtained.

\[
\% \text{ of moisture content} = \frac{\text{Loss in weight of sample}}{\text{Weight of sample}} \times 100
\]

**Fluorescence analysis**

Fluorescence analysis was carried out by treating the powdered drug with different reagents, namely ethanol (75% v/v), ethyl acetate, acetone, methanol, chloroform, carbon tetrachloride, water and petroleum ether and observed at 254 nm, 366 nm in a UV chamber and visible light.

**Leaching degree**

Leaching degree was determined by treating the powdered drug with different reagents, namely ethanol (75% v/v), ethyl acetate, acetone, methanol, chloroform, carbon tetrachloride, water and petroleum ether. Filtered and filtrate was evaporated to obtained dried extract.

\[
\% \text{ of leaching degree} = \frac{\text{Weight of dried extract}}{\text{Weight of sample}} \times 100
\]

**Thin Layer Chromatography**

Thin Layer Chromatography studies were carried out for themethanolic-petroleum ether extract of *P. rubra* and reference sample Rutin. The spots obtained from both the extracts were examined under ultraviolet light, of wavelengths 365 nm.

An aluminum plate (20×10 cm) precoated with CMC-Na(0.5%)-silica gel GF254 was used as the absorbent. The solvent system was chloroform: methanol: water: formic acid(7:3:1:0.1). The methanolic-petroleum ether extract of *P. rubra* was prepared by using 5 g powder, treated with 100 mL of methanol, filtered and decolor with petroleum ether. The plate was developed in a Camag twin trough chamber and examined at 365 nm.

**RESULTS**

**Macroscopic characters**

*Fresh plant:* *P. rubra* is a shrub or small tree with a height of 0.5-5 meters tall. Leaves are elliptic-oblong, lanceolate-oblong, or rarely oblong-ovate in shape and opposite each petiole, drying papery to leathery, measuring 5-23.5 cm long and 2-9 cm wide with entire margin and acute apex. The color is green when fresh, while in dry condition, upper surface is yellowish green or gray-green color whereas underside is dark red or brownish red color. Petiole is glabrous or rarely puberulent. Stipules are membranous and caducous with short sheath shape, broadly rounded to obtuse or shallowly emarginate (Figure 1A).

The inflorescence is terminal or sometimes pseudoaxillary, cymose to panículiform, many-flowered. Flowers are glabrous or usually densely puberulent, sessile and apparently tripartite to shortly pedunculate. Peduncle is very short about 0.3 mm and branched portion is corymbiform-rounded to broadly pyramidal, with 1-3 pair(s) of developed secondary axes. Corolla is white with funnel form, glabrous outside (Figure 1B).

Fruits are drupes which are red in color, subglobose to broadly ellipsoid with a length of 5-8 mm, diameter 4-7 mm, with pedicels to 10 mm (Figure 1C).

*Dried plant:* Stems are brownish black in color with cylindrical shape, easy to break up by hand. Fracture surface is not flat and fibrous. It has an mild aromatic odor and a light taste. Leaves are oblong in shape and bronzing or pinky in color with no wrinkle, lenticel and plash whereas with membranous leaves (Figure 1D).
Microscopic characters

**Epidermis:** The upper epidermis cells are squarish-shaped form without nonglandular hairs and stomata (Figure 2A), while the lower epidermis cells are polygonal-shaped form with stomata, which are paracytic type, surrounded by two to three subsidiaries (Figure 2B).

**T.S. of root:** Transverse section of root is circular in outline consisting of epidermis, cortex, and vascular zone. The epidermis is broken and obliterated. Cork layer is incorporated in many layers of cells and phelloderm is not conspicuous. Fiber bundles and stone cells are scattered in cortex, with thin walled parenchymatous cells surrounded by calcium oxalate crystal. The secondary phloem is narrow, made up of polygonal-shaped cells, densely arranged. Xylem is surrounded by interfascicular cambium which is less distinct. Xylem rays are conspicuous, and the vessels are circular, most of which are arranged in single or V-shaped (Figure 3).

**T.S. of stem:** Transverse section of stem is oblong in outline consisting of epidermis, cortex and well-developed vascular zone. Cork layer is cuticularized and strongly lignified with a layer of tidily and closely arranged cells and a thicker wall. Lenticels can be seen occasionally and phello-
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**Powder analysis:** Microscopic study of powder (Figure 6) reveals the presence of stone cells, cork cells, needle crystals, resin duct, pollen grains, tracheid and vessels. Most of vessels are spiral shaped ranges the length from 31.2-56.4μm. Cork cells are rectangle in shape with thick wall. Stone cells are rare and oblong-like shaped with thicker wall and obvious cinclides. Fibers are multiple gathered, yellowish green in color and slender in shape with thick wall and obvious pits. Needle crystals are smaller and long fusiform with blunt end, diffused into single one or fascicles.

**Phytochemical screening**

Preliminary qualitative phytochemical screening of water and petroleum ether soluble extract of *P. rubra* showed the presence of carbohydrates, saponins, tannins and volatile oils. Ethanolic extract showed the presence of flavones, while acid-water soluble extract showed alkaloids (Table 1).

**Physio-chemical analysis**

Physio chemical analysis revealed that moisture content is 1.28 %, total ash content is 9.58% and acid insoluble content is 0.65 %, respectively(Table 2).

**Fluorescence analysis**

Fluorescence analysis of powder and different extracts of *P. rubra* with different reagents were carried out to observe the color reactions (Table 3).

**Leaching degree**

Leaching degree results were tabulated in table 4, which revealed that leaching degree of ethanol(75%, v/v) and methanol extracts from *P. rubra*were much higher, whereas much lower in chloroform, petroleum ether and carbon tetrachloride extracts(Table 4).
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**Figure 6:** SC: stone cells; CC: cork cells; NCB: Needle crystal bundles; RC: resin canal; PG: pollen grains; Tr: tracheid; Ve: vessels.

**Figure 7:** TLC of *P. rubra*: A1, A2, A3-sample, B-standard.

Table 1: Preliminary phytochemical screening of *P. rubra*

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Test</th>
<th>Observations</th>
<th>Presence(+)/absence(-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar</td>
<td>Molisch’s test</td>
<td>Purplish-red ring appeared</td>
<td>+ve</td>
</tr>
<tr>
<td>Glycosides</td>
<td></td>
<td>No Purplish-red or blue color</td>
<td>-ve</td>
</tr>
<tr>
<td>Amino acid</td>
<td>Ninhydrin test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polypeptide</td>
<td>Froth formation test</td>
<td>Lots of froth formed for 10 min</td>
<td>+ve</td>
</tr>
<tr>
<td>Proteins</td>
<td>FeCl₃ test</td>
<td>Dark green color appeared</td>
<td>+ve</td>
</tr>
<tr>
<td>Saponins</td>
<td>Filter paper test</td>
<td>Oil spot appeared</td>
<td>+ve</td>
</tr>
<tr>
<td>Tannins</td>
<td>Hydrochloric acid - Mg test</td>
<td>Red forth</td>
<td>+ve</td>
</tr>
<tr>
<td>Volatile oil and fats</td>
<td>Acetic anhydride - concentrated sulfuric acid test</td>
<td>Yellow fluorescence enhanced</td>
<td>+ve</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Lye test</td>
<td>Black precipitate</td>
<td>-ve</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>Dragendorff’s test</td>
<td>Pale yellow precipitate</td>
<td>+ve</td>
</tr>
<tr>
<td>Phytosterols</td>
<td>Iodine - potassium iodine test</td>
<td>White precipitate</td>
<td>+ve</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>Silicotungstic acid test</td>
<td>Brown precipitate</td>
<td>+ve</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Phosphomolybdic acid test</td>
<td>No Pale yellow or white color</td>
<td>-ve</td>
</tr>
</tbody>
</table>

**Thin Layer Chromatography**

The TLC profiles of *P. rubra* extract and Rutin standard were obtained under UV 365nm light after post-derivatization with AlCl₃. Figure 7 showed the flavone spot in *P. rubra* extract specifically (Figure 7). Distinct TLC spot on the silica gel plate representing isolated compound with specific Rf values (Rf = 0.47).

**DISCUSSION AND CONCLUSION**

The standardization of a crude drug is of key importance in establishing its proper identity, also plays an imperative role in guaranteeing the botanical quality and clinical efficacy. Before any crude drug can be included in a herbal pharmacopoeia, it is prerequisite to establish pharmacognostic parameters and standards based on macro and microscopic evaluation as well as detection of adulterants and substitutes. However, the leaves, stems and roots of this plant were used by local people for the treatment of various disease without standardization. The study provided some basic data regarding the genuine crude drug. The stone cells, calcium oxalate crystal, vessels, lenticels, crystal and resin, fibers, needle crystal beam, thread tracheid and scalariform tracheid were observed under the microscope which were used as ingredients. All the physicochemical parameters such as total ash value, acid insoluble value and moisture content were analyzed and found to be within limit mentioned by Pharmacopoeia of China. Fluorescence analysis and thin layer chromatography, like parameters above, are unique to the plant and are required in its standardization. Such information of the pharmacog-
Pharmacognostical evaluation may be useful to identity of *P. rubra* which may act as reference information and produce a solid basis for proper identification, authentication, collection and investigation of the plant material. Further, it will be helpful for detecting adulterants and substitutes and maintaining the quality, reproducibility and efficacy of natural drugs.

**ACKNOWLEDGEMENT**

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**REFERENCES**


**GRAPHICAL ABSTRACT**

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**SUMMARY**

- The present study was carried out to made a detailed investigation on pharmacognostic parameters of *Psychotria rubra* (Lour.) Poir to help in identification and standardization of this drug.
- Morphological and microscopic characters of the roots, stems and leaves of the plant were observed. The histochemistry and physicochemical parameters were studied including ash value, moisture content, leaching degree, fluorescence analysis and Thin Layer Chromatography.
- T.S. of root showed stone cells, calcium oxalate crystal and vessels in circular shape. T.S. of stem showed lenticels and stone cell bands. T. S. of leaf vein revealed the presence of shaft type of porosity. Powder microscopy revealed the presence of cork cells, stone cells, crystals and resin, fibers, needle crystal beam, thread tracheid and scalariform tracheid.
- Phytochemical investigation was found like alkaloids, flavones, carbohydrates, saponins, tannins and volatile oils. Physicochemical analysis showed total ash value, acid soluble ash and moisture content were 9.77%, 0.68% and 1.40% respectively. Fluorescence analysis and Thin Layer Chromatography were unique to the plant and required in its standardization.

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**Table 2: Physicochemical parameters of *P. rubra***

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Results (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture content</td>
<td>1.28±0.13%</td>
</tr>
<tr>
<td>Total ash content</td>
<td>9.58±0.085%</td>
</tr>
<tr>
<td>Acid insoluble content</td>
<td>0.65±0.035%</td>
</tr>
</tbody>
</table>

**Table 3: Fluorescence analysis of *P. rubra***

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Visible/Day Light</th>
<th>UV 254 nm</th>
<th>UV 366 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>Pale pink</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Ethanol(75%)</td>
<td>Sepia</td>
<td>Reddish brown</td>
<td>None</td>
</tr>
<tr>
<td>Acetone</td>
<td>Olive green</td>
<td>Bright red</td>
<td>Pale red</td>
</tr>
<tr>
<td>Methanol</td>
<td>Dark green</td>
<td>Pinkish red</td>
<td>Dark red</td>
</tr>
<tr>
<td>Chloroform</td>
<td>Yellowish green</td>
<td>Pale red</td>
<td>Reddish brown</td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>Pale green</td>
<td>Pale pink</td>
<td>Pale red</td>
</tr>
<tr>
<td>Carbon tetrachloride</td>
<td>Pale green</td>
<td>Pale brown</td>
<td>Pale red</td>
</tr>
</tbody>
</table>

**Table 4: Leaching degree of *P. rubra***

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Leaching degree (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>12.6</td>
</tr>
<tr>
<td>Ethanol(75%)</td>
<td>26.5</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>6.60</td>
</tr>
<tr>
<td>Acetone</td>
<td>10.8</td>
</tr>
<tr>
<td>Methanol</td>
<td>21.5</td>
</tr>
<tr>
<td>Chloroform</td>
<td>1.40</td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>0.60</td>
</tr>
<tr>
<td>Carbon tetrachloride</td>
<td>1.47</td>
</tr>
</tbody>
</table>
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