Indian Journal of Traditional Knowledge
Vol 20(2), April 2021, pp 370-377

Pharmacognostical, phytochemical and physiochemical studies of *Bridelia tomentosa* Bl.

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Received 17 June 2019; revised 12 January 2021

To provide a scientific basis for identification of *Bridelia tomentosa* Bl., the current study was carried out for pharmacognostical and preliminary phytochemical analysis. The roots, stems and leaves were used for character identification, microscopic identification and physicochemical parameters analysis which mainly included the commonly used methods, like ash value, UV absorption spectrum and thin layer chromatography (TLC) etc. Pharmacognostic studies revealed presence of non-glandular hairs in leaf midrib transverse section, calcium oxalate cluster crystals and calcium oxalate crystals in stem transverse section, fibers in root transverse section, cork cells, fibers, non-glandular trichomes, pitted vessels, calcium oxalate crystals and calcium oxalate crystal clusters in powder microscopy. Phytochemical investigation showed the presence of carbohydrates, tannins and volatile oils from water and petroleum ether soluble extract, while flavones, phytosterol and triterpene were detected from ethanol extract and alkaloids from acid-water soluble extract. The presence of chlorogenic acid in *Bridelia tomentosa* extract with specific Rf values was detected using TLC. The findings confirmed that *Bridelia tomentosa* clearly has significant pharmacognostical characters, which would be valuable for providing a dependable foundation for authenticity of plant materials.

Keywords: *Bridelia tomentosa*, Identification, Physicochemical analysis, Phytochemical screening

IPC Code: Int Cl.[21]: A23F 3/14, A23F 3/18, A23F 3/22, G16C 20/40, B32B 7/02, A01G 7/00

Bridelia tomentosa Bl., a traditional medicinal plant locally known as "Tumishu" in China, is a small shrub that belongs to genus Bridelia, family Euphorbiaceae. It is extensively distributed in Southeast Asia including Indonesia, Malaysia, Australia and China. Normally it grows in mountainous forests or plain shrubbery at 100-1500 m altitude[1].

As a traditional Chinese medicine, leaves and leaved branches of *Bridelia tomentosa* are used for the treatment of trauma bleeding and bruising injury, while it's root is used for curing neurasthenic and menoxenia. Usually 8.08% tannin can be extracted from bark of *Bridelia tomentosa*[1]. Besides, a decoction of leaves is used for colic or high fever in Thailand[2] and root is used to treat liver dysfunction in India[3].

The phytochemical composition and various pharmacological activities of *Bridelia tomentosa*'s extracts were determined in previous studies. A new triterpenoid obtained from the roots of *Bridelia tomentosa* was identified as 24-methyllanosta-9(11), 25-dien-3-one2 and two new flavonol glycosides elucidated as tamarixetin 3-O-β-D-xylopyranosyl-(1-2)-α-L-ribopyranoside and tamarixetin 3-O-α-L-ribopyranoside were isolated from the leaves[4]. Besides, ten compounds were identified in the fruits of *Bridelia tomentosa*[5].

To our best knowledge, most previous studies focused on its pharmacological activities and chemical compositions, but little attention has been paid to its pharmacognostical characters. Yet the report on pharmacognostical studies of *Bridelia tomentosa*'s roots was not complete and unsystematic[6]. Therefore, the information regarding its pharmacognostical identification on the leaves, stems and roots is still very scanty and poorly understood.

With huge digging value and beneficial value, it is of great significant to research comprehensively and precisely about its pharmacological properties. On the basis of the earlier studies of plant anatomy,

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herein we made a detailed investigation on macroscopy, microscopy, phytochemical screening, physicochemical parameters, powder behavior, fluorescence analysis, UV analysis and thin layer chromatography of the whole plant to certify its authenticity and standardization.

Methodology

Collection and identification of plant material

The plants were collected on 17 July 2018 from Guangzhou in China (23°3′30″N 113°24′32″E), taxonomically identified as *Bridelia tomentosa* Bl., Euphorbiaceae by Prof. Shengguo Ji, School of Traditional Chinese Medicine, Guangdong Pharmaceutical University. The voucher specimen number is TMS/01/17072018. The plants were dried after washing, then powdered with a mechanical blender and passed through sieve no. 65 for the study.

Preparation of sample

Transverse section

By using standard procedures, the fresh roots, stems and leaves of *Bridelia tomentosa* Bl. were sliced for hand cutting. These were cut into segments about 3 cm long. In addition, 1 cm wide leaves were fixed in FAA (formalin 5 mL + Acetic acid 5 mL + 70% Ethyl alcohol 90 mL) for microscopic examination[7].

Leaf epidermis

About 2-3 fresh leaves were taken and tweezers were used to directly tear the upper and lower epidermis of the leaves. The torn epidermis was sliced into petri dishes with distilled water and the cells and stomata were observed. The calculation formula of stomatal index is as follows[8].

$$\% \text{ of Stomatal Indices} = \frac{\text{Stomatal number per unit area}}{\text{Stomatal number per unit area} \times \text{Epidermal cell number of same area}} \times 100\%$$

Powder

The appropriate amount of fresh samples were dried and pulverized to powder using a mill and passed through a 65-mesh sieve. About 0.1 g powder was put under coverslip with 50% glycerin, then heated and permeated in chloral hydrate solution (10%, v/v), followed by microscopic examination, in order to obtain information about morphology and powder characteristics, stone cell, non-glandular trichomes, calcium oxalate cluster crystal, etc.

Macroscopic characters

The macroscopic appearance of fresh plants was evaluated by observing their colour, shape, size, texture and broken face.

Microscopic characters

Histological study was carried out by exploring powder microscopically and immobilizing the fresh material with FAA. The fine sections of roots, stems and leaves were dehydrated under a series of ethanol concentrations and then dyed with saffron and fixed with neutral resin. The free hand part of the Motic Multi-plexer was observed on the Nikon eclipse E100 microscope with high resolution. Drawing support from image processing application software Finial View, the photograph was taken under visible light. All basic but essential features were tested and recorded appropriately.

According to the standard guidelines, the phytochemical and physicochemical parameters of the powder were analyzed. Phytochemical test, ash content (total ash and acid insoluble ash), water content, powder drug behavior, fluorescence, UV spectrum and TLC analysis were included[9-11].

Phytochemical tests

For preliminary phytochemical screening, dried, coarsely powdered material of *Bridelia tomentosa* (3 g) was extracted successively with water, petroleum ether, 75% ethanol and 0.5% hydrochloric acid solution for 30 min. Several qualitative chemical tests were conducted for the identification of various active constituents viz., triterpenoids, steroids, alkaloids, sugar, tannins, glycosides and flavanoids, etc.

Total ash value

The 3 g powdered sample was further weighed in a silica crucible to incinerate till carbonless ash. The temperature was raised to 500-600 gradually till a constant value was obtained. The total ash value was calculated by the following formula:

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

Acid insoluble ash

About 10 mL dilute hydrochloric acid was measured and added to the total ash obtained above in a silica crucible, which was covered with watch glass and was boiled for 10 min. It was then filtered and residue was washed until the lotion did not show an

oxide reaction, dried and burn until a constant value was obtained. The acid-insoluble ash value was obtained by the following formula:

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

Moisture content

Moisture content of powder was determined by weighing 2-5 g of the powder sample in a weighing bottle, which was dried to constant weight, then the weighing bottle was placed in a hot air oven at 105 for 1 h until constant weight of the sample was obtained. The moisture content is calculated according to the following formula:

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

Behavior of powder

The powder was treated with different reagents namely glacial acetic acid, sulfuric acid, sodium hydroxide, potassium hydroxide, ferric chloride, hydrochloric acid and nitric acid. The behavior of powder was observed by floating or dropping and the changes of solution colours.

Fluorescence analysis

Various chemical reagents were used for ultrasonic processing of the powder and the fluorescence analysis was carried out under visible light, 254 nm and 365 nm in a UV chamber.

UV absorption spectrum

About 2 g of powder was weighed and separately treated with 30 mL of ethanol (95% v/v), ethyl acetate and chloroform by ultrasonic processing, filtered and 2 mL of filtrate was transferred to a 50-mL volumetric flask, diluted with water to desired volume and mixed for UV analysis. The UV absorption spectrum of text sample and standards ethanol (95% v/v), ethyl acetate and chloroform were separately determined at a wavelength of 200-800 nm.

Thin layer chromatography (TLC) fingerprint profiles

Firstly, the crude extract of *Bridelia tomentosa* was dissolved in methanol. Secondly, the methanol extract of *Bridelia tomentosa* and the standard solution chlorogenic acid were studied by TLC. The spots of the two extracts were detected by UV at 365 nm using aluminum plate (10×20 cm) consisting of Silica gel Gas stationary phase and ethyl acetate: water: methanol (8:1:1) as the mobile phase. The phenomenon of the thin plate was observed after developing in developing tank with temperature of 24 and relative humidity of 49%, detecting under the ultraviolet light of 365 nm wavelength, then spraying with aluminum chloride developer, finally heating at 105.

Results

Macroscopic characters

Fresh plant

Bridelia tomentosa is a small shrub or arbor with a height of 2-5 m tall and bark in dark gray. Branches are slender with petiole measuring 3-5 mm. All parts of the plant are glabrous except for cladium, blade back, petiole, stipule and outer sepal of pistillate flower. Leaf blades are papery with ovale, oblong or obovate-ovale in shape, measuring 3-9 cm long and 1.5-4 cm wide. Apex is acute to obtuse while base is broadly cuneate to subrotund in shape with rough leaf surface and blade back in laurel-green. Flowers are monoecious or gynodioecious with almost year-round fruiting period. Drupe is spheroidal in shape (Fig. 1a).

Dried plant

The stem is taupe and easy to be broken by hands. Leaves are slightly wrinkled, papery and glabrous both sides in oblong shape after being unfolded. Apex is acute to obtuse while base is broadly cuneate in shape with rough leaf surface and blade back in laurel-green. It has a mild smell and a slightly astringent taste (Fig. 1b).

T.S. of Root

The transverse section of the root is round, which is composed of epidermis, cortex and vascular zone. The cork layer consists of 3-5 layers of relatively compressed cells with obvious phelloderm. Cortex is wider with many fiber bundles in oval scattered. The

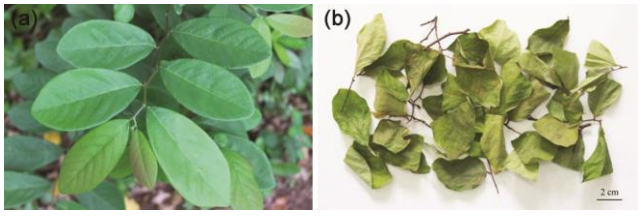

Fig. 1 — (a): Fresh plant of *Bridelia tomentosa* (b): Dried plant of *Bridelia tomentosa*

cambium is annular, with a narrow phloem and the well-developed and wide xylem, whose vessels are monodispersed or clustered (Fig. 2).

T. S. of stem

The transverse section of the stem is similar to the root. But the cork layer is relatively thicker than other layers of cells. Phelloderm is made up of 2-3 layers of collenchymatous cells in polygonal shape. Cortex is wider with fiber bundles in oval scattered and surrounded by calcium oxalate square crystals. Phloem is narrow with cambium formed into a ring. Vessels are arranged radially in the xylem, of which cell walls are lignified. At center, pith is broad with calcium oxalate crystals and calcium oxalate cluster crystals (Fig. 3).

T.S. of leaf midrib

The leaf epidermis is composed of a layer of guard cells with non-glandular trichomes outside of the lower epidermis. The mesophyll tissue was divided into two kinds: palisade parenchyma composed of 1-2 layers of cylindrical cells and the spongy tissue cells loosing the larger gap. The xylem is lignified with radial vessels, arranged in a concave shape and surrounded by narrow phloem. Fibre bundles are compactly arranged in a ring around the xylem and phloem, while vascular bundles are shaped in a deep groove (Fig. 4).

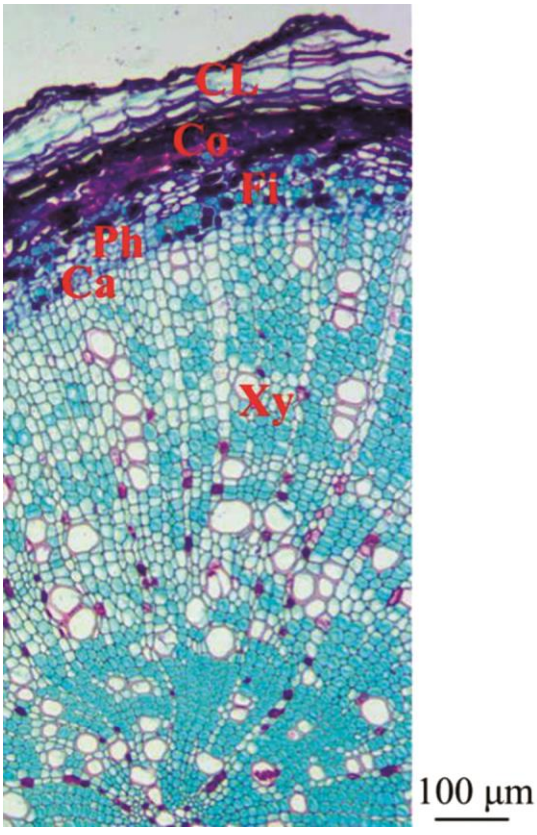

Fig. 2 — T.S. of Root of *Bridelia tomentosa*. CL: cork layer; Co: cortex; Fi: fiber; Ph: phloem, Ca: Cambium; Xy: xylem.

Epidermis

The upper epidermis cells with straight anticlinal wall are polygonal-shaped with stomas, which are mostly paracytic type, surrounded by two subsidiary

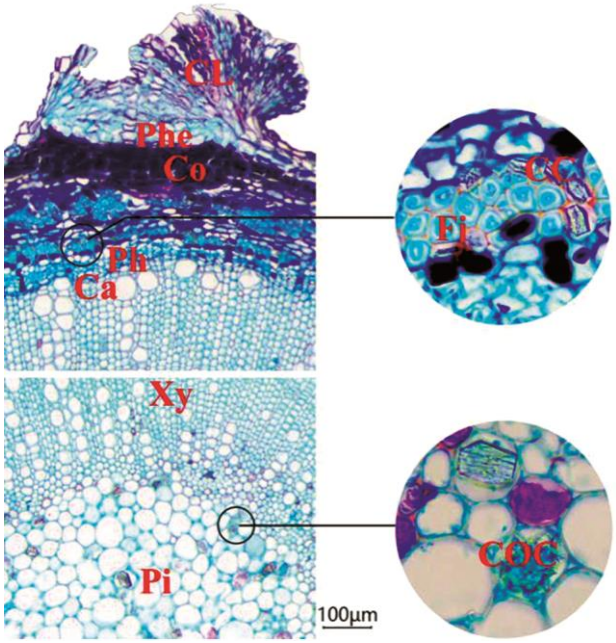

Fig. 3 — T.S. of Stem of *Bridelia tomentosa*. CL: cork layer; Phe: Phelloderm; Co: cortex; CC: calcium oxalate crystals; Fi: fiber; Ph:phloem; Ca: Cambium; Xy: xylem; COC: calcium oxalate cluster crystals; Pi: pith.

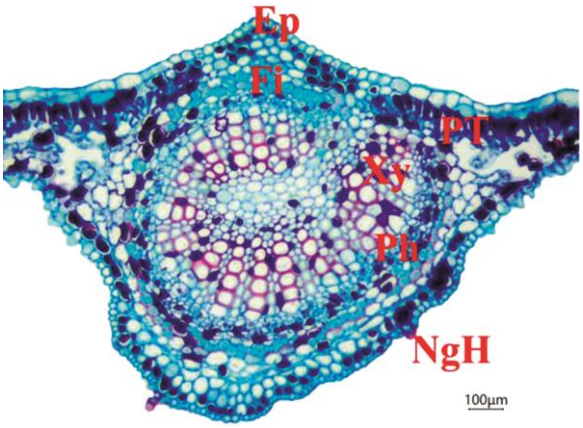

Fig. 4 — T.S. of leaf midrib of *Bridelia tomentosa*. Ep: epidermis; Fi: fibers; PT: palisade tissue; Xy: xylem; Ph: phloem; NgH: nonglandular hairs.

cells with stomatal index at 7.9 % (Fig. 5a). Similarly, the lower epidermis cells with wavy anticlinal wall are erose-shaped form with stomas, which are mostly paracytic or irregular, surrounded by two to five subsidiary cells with stomatal index at 15.8 % (Fig. 5b).

Powder

Observation under the microscope (Fig. 6) shows presence of cork cells, non-glandular trichomes, calcium oxalate crystals and cluster crystals, fibers, pitted vessels, etc. The yellowish brown and quasi-polygonal cork cells have straight and thick outer walls. Fibers are yellowish brown coloured in straight or slightly curve shaped with 7-29 µm in diameter and the cells around the fiber bundles are with presence of calcium oxalate crystals forming crystalline fibers. Most of vessels are pitted shaped with obvious pit aperture and range with 6-24 µm in diameter. Non-glandular trichomes composed of many cells are curly and thick, measuring 70-130 µm long and with 5-14 µm in diameter. Calcium oxalate cluster crystals are mostly diffuse into a single one with blunt end, while calcium oxalate crystals are less and in rhombus shape diffused into single one.

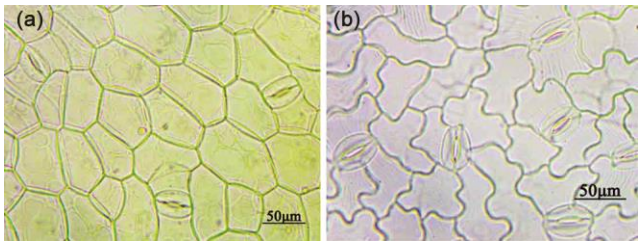

Fig. 5 — Epidermis of leaf of *Bridelia tomentosa*. a: Upper epidermis (UE); b: Lower epidermis (LE).

Phytochemical screening

Preliminarily qualitative phytochemical screening of various extracts of *Bridelia tomentosa* showed the presence of carbohydrates, tannins and volatile oils. Ethanol extract showed the presence of flavones, phytosterol and triterpene, while acid-water soluble extract showed alkaloids (Table 1).

Physio-chemical analysis

Physio-chemical analysis revealed that moisture content is 10.74%, total ash content is 6.54% and acid insoluble content is 0.42%, respectively (Table 2).

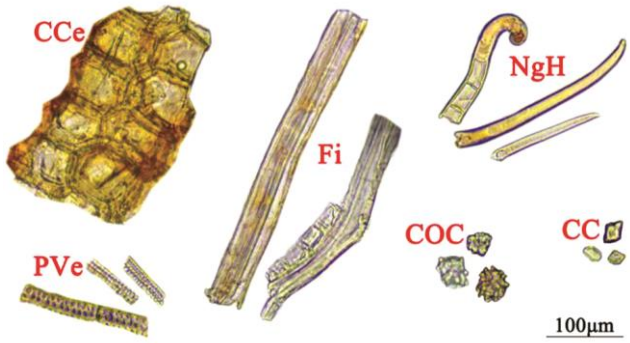

Fig. 6 — Powder microscopy of *Bridelia tomentosa*. NgH: nonglandular hairs; CCe: cork cells; PVe: pitted vessels; Fi: fibers; CC: calcium oxalate crystals; COC: calcium oxalate cluster crystals.

Table 1 — Phytochemical screening of *Bridelia tomentosa*

Phytochemical	Test	Observations	(+)/(-)
Sugar Glycosides	Molisch's test	Purplish-red ring	+
Polypeptide Proteins	Ninhydrin test	No phenomena	-
Saponins	Froth formation test	Little forth formed but disappear	-
Tannins	$FeCl_3$ test	Green colour	+
Volatile oil and fats	Filter paper test	Oil spot	+
Flavonoids	Hydrochloric acid - Mg test	Red forth	+
	Aluminum trichloride test	Yellow fluorescence	+
	Ammonia smoked test	Yellow fluorescence	+
Anthraquinones	Lye test	No phenomena	-
Phytosterols triterpenoids	Acetic anhydride -concentrated sulfuric acid test	Colour changing	+
Cardenolide	Baljet test	No phenomena	-
Alkaloids	Dragendorff's test	Yellow precipitate	+
	Iodine - potassium iodine test	Brown precipitate	+
	Silicotungstic acid test	White precipitate	+
	Phosphomolybdic acid test	White precipitate	+

(Here: (+) is presence, (-) is absence)

Behavior of powder

After treating with different reagents, studies on different appearances and solution colours were carried out by powder analysis (Table 3).

Fluorescence analysis

Different reagents were used for fluorescence analysis of *Bridelia tomentosa* powder and different extracts and the colour reaction was observed (Table 4).

Table 2 — Physicochemical parameters of *Bridelia tomentosa*

Sr. No	Parameters	Results (w/w)
1	Moisture content	10.74%±0.11%
2	Total ash content	6.54%±0.075%
3	Acid insoluble content	0.42%±0.015%

Table 3 — Behavior of powders of *Bridelia tomentosa*

Regent	Appearances of powders	Solution colour
Hydrochloric acid	Floating	Green
Sodium hydroxide	Floating	Yellowish brown
Ferric trichloride	Floating	Yellowish green
NitricG:\Youdao\Dict\ 7.5.2.0\resultui\dict\? keyword=acid	Floating	Orange
Potassium hydroxide	Floating	Yellowish brown
Sulfuric acid	Charry	Dark green
Glacial acid	Depositing	Green

Table 4 Fluorescence analysis of *Bridelia tomentosa*

Reagent	254 nm (UV)	365 nm (UV)	Visible Light
H_2O	None	None	Yellowish green
C_2H_5OH (75%)	Red	Shiny red	Green
Ethyl acetate	Pink	Shiny red	Green
Acetone	Red	Shiny red	Dark green
Methanol	Red	Red	Green
Chloroform	Pink	Pink	Dark green
Petroleum ether	Pink	Shiny red	Green
Carbon tetrachloride	None	Pinkish red	Green

UV absorption spectrum

UV analysis revealed that the values of strong absorption obtained from 95% ethanol extract, ethyl acetate extract and chloroform extract were (412 nm, 664 nm), (408 nm, 666 nm) and (666 nm), respectively (Fig. 7).

Thin Layer Chromatography

Both the TLC fingerprint of *Bridelia tomentosa'*s methanol extract and chlorogenic acid standard were obtained under UV light of wavelength 365 nm. Figure 8 showed the chlorogenic acid spot in *Bridelia tomentosa* extract specifically. Distinct TLC spot on the silica gel plate representing isolated compound with specific R_f values (R_f = 0.61).

Discussion

Establishing standards is a crucial part of determining the identification, purity, quality and safety of a crude drug. It is essential for preventing adulteration by giving proper information about the source plant material. If not, misidentification may directly be hazardous to human health[12]. It is of great

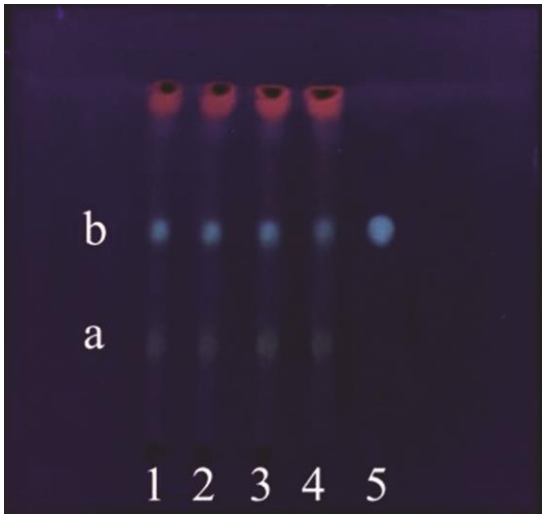

Fig. 8 — TLC of *Bridelia tomentosa*. Lanes1-4- sample; Lane 5- standard.

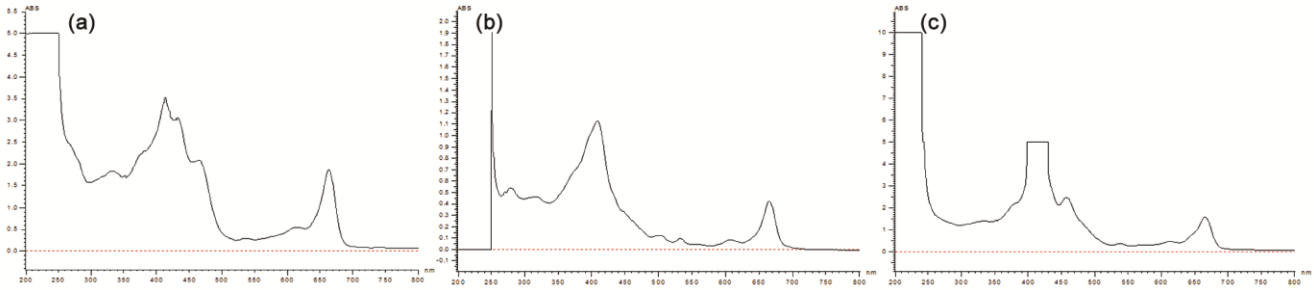

Fig. 7 — UV absorption spectrum of *Bridelia tomentosa*. A: 95% ethanol extract; B: ethyl acetate extract; C: chloroform extract.

importance to improve the standardization level of TCM to evaluate pharmacognosy by means of macroscopic, microscopic and physicochemical analysis. However, in the modern medical system, there is not enough evidence for the standardization of *Bridelia tomentosa* due to little pharmacognostical or anatomical work being explored to it. Considering these facts, the study has tried to find out the pharmacognostic characteristics of the plant in order to identify and standardize the plant materials.

Evaluation of the macroscopic appearance of the plant, including taste, sight, smell and touch, as a first step, can be easily employed and contributes to their correct identification and quality. Microscopic studies, whose main features are cork cells, fibers, ducts and non-glandular hairs, are reliable, simple and cheapest in establishing the identity of source materials[13].

Extractive values are used in qualitative as well as quantitative estimation of phytoconstituents which act as preliminary information and show the sign of adulteration or deterioration about the drug[14]. All the physicochemical parameters such as total ash value, acid insoluble value and moisture content were analyzed and found to be within limits mentioned by Pharmacopoeia of China. All of these may facilitate the identification of formulations in routine industrial production.

The preliminary phytochemical analysis in the plant extract showed various types of bioactive compounds like carbohydrates, tannins, volatile oils, flavonoids, phytosterols, triterpenoids and alkaloids, which make the plant pharmacologically and therapeutically active. Presence of these constituents may be responsible for its usefulness in the treatment of many ailments and possible to demonstrate the sites of production or accumulation of some metabolites of the plant[15].

As a plant-specific parameter, fluorescence analysis shows the marker of chromophore in plant. Fluorescence analysis can be utilized in evaluation to check its quality, purity or presence of some chemical constituents in the first-line standardization[16]. Many sections or powders show fluorescence when exposed to UV and it may be helpful to further detect the adulteration.

TLC is an important technique by which the quality standardization and fingerprint of plant drug can be sustained. They also help to identify the individual herbs in poly-herbal formulations[17]. The main purpose of TLC evaluation of *Bridelia tomentosa* is to determine whether chlorogenic acid is contained.

Above all, the results of this study provided a solid foundation for the identification and characterization of *Bridelia tomentosa*. However, all investigations are preliminary and more advanced technology should be used to research phytochemicals with different pharmacological activities. Therefore, more attention should be paid to the development of scientific research on this plant.

Conclusion

In the current study, evaluation of macroscopy, microscopy, phytochemical screening and physicochemical parameters were carried out and this could be helpful in authentication of *Bridelia tomentosa*. Futhermore, the results may provide experimental evidence for the fundamental understanding of *Bridelia tomentosa* and aid the development of further experiments. Above all, this comprehensive research will be useful to authenticate, standardize and distinguish adulteration in the plant material.

Acknowledgement

All authors are most grateful to Professor Shengguo Ji for guidance and encouragement. They also express sincere appreciation to Guangdong Pharmaceutical University for providing them with necessary facilities.

Conflict of Interest

All authors declare no competing or conflict of interest.

Authors Contribution

Conceptualization: JFW, XJL, GQT; Formal analysis: JFW, JNL, RL; Funding acquisition: SGJ, DW; Resources: JFW, XJL, SGJ; Software: JFW, XJL, GQT, JNL, CBZ; Supervision: JFW, GQT, JNL HJG, WFW; Roles/Writing - original draft: JFW, XJL, GQT; Writing - review & editing: JNL, CBZ, HJG, WFW, SGJ, DW;

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