ESTABLISHING A PRELIMINARY QUALITY STANDARD FOR *HERBA MIMOSAE PUDICAE*

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Abstract: *Mimosa pudica L. (commonly known as "Xấu hổ" in Vietnam) is a widely used medicinal plant and serves as a raw material for various traditional medicine formulations due to its anti-rheumatic and sedative properties. However, the Vietnamese Pharmacopoeia V has not yet established a monograph specifying quality standards for this herbal material. This study aimed to develop an in-house quality standard for Herba Mimosae pudicae, including macroscopic and microscopic characterization (plant anatomy and histology); determination of moisture content; total ash; qualitative identification by thin-layer chromatography (TLC); powder microscopy analysis; acid-insoluble ash; and quantitative determination of the principal active constituent using high-performance liquid chromatography (HPLC). The medicinal material Mimosa pudica L. was characterized in terms of macroscopic and microscopic features. TLC analysis showed spots with identical color and R_f values compared to those of the reference standard. The mimosa content ranged from 264 to 364 µg/g. Quality parameters were established as follows: moisture content < 13%, foreign matter < 1%, and total ash < 8%. This study has preliminarily established a set of in-house quality standards for the medicinal plant Mimosa pudica L.*

Keywords: *Mimosa pudica L., mimosine quantification, herbal material standards, HPLC analysis.*

1. INTRODUCTION

Mimosa pudica L. (commonly known as "Xấu hổ" in Vietnam) belongs to the family Fabaceae. It is also known by other vernacular names, including "Trinh nữ", "Mắc cỡ", and "Hàm tu thảo" (Figure 1). The plant is characterized by bipinnately compound leaves that fold rapidly in response to touch, small pinkish-purple flowers arranged in globose heads, and segmented pods containing oval seeds [1]. It commonly grows in grasslands, roadsides, and other open areas, particularly on dry soils. The plant flowers from June to October and fruits from October to January of the following year [1].

The whole plant (*Herba Mimosae pudicae*) is used medicinally. It has a sweet and astringent taste, is slightly cold in nature, and exhibits sedative, analgesic, expectorant, antitussive, antipyretic, anti-inflammatory, and diuretic effects [2]. It is commonly used to treat neurasthenia, insomnia, and musculoskeletal pain [1].

Figure 1. *Mimosa pudica L. plant.*

Phytochemical investigations have revealed the presence of various bioactive constituents, including alkaloids, flavonoids, tannins, saponins, terpenoids, glycosides, and phytosterols in different parts of the plant [3 – 5]. Among these compounds, mimosine is considered a characteristic constituent and has been widely used as a chemical marker for *M. pudica* [4]. Previous pharmacological studies have demonstrated anxiolytic, antidepressant, anticonvulsant, memory-enhancing, antibacterial, anti-inflammatory, and anticancer activities of extracts and isolated compounds from this species [6 – 12].

Although *Herba M. pudicae* is widely used in traditional medicine formulations in Vietnam, it has not yet been included as a monograph in the *Vietnamese Pharmacopoeia V*. Therefore, this study was conducted to develop in-house quality standards for *Herba Mimosae pudicae*.

2. EXPERIMENTAL

2.1. Study materials

The investigated material was the whole plant of *Mimosa pudica* L. (sample code: XH_01.23), belonging to the family Fabaceae. The plant material was washed, cut into small pieces, and dried at 60°C in a vacuum drying oven. The dried material was stored in polymer bags under dry, well-ventilated conditions to prevent moisture absorption and mold growth. The reference standard of *M. pudica* was provided by the Center for Ginseng and Medicinal Materials, Ho Chi Minh City.

Apparatus: HPLC analysis was carried out using a Shimadzu LC-2030C 3D Plus system (Japan) equipped with high-pressure pumps, an autosampler, and a PDA detector. A Shim-pack GIST C18 column (250 × 4.6 mm, 5 µm) coupled with a C18 guard column (10 × 4.6 mm, 5 µm) was used for separation. Other instruments included a dual-wavelength UV lamp (254/365 nm; CN-15-LC, Vilber Lourmat, France), a JSM-IT100 scanning electron microscope (JEOL, Japan), ...

2.2. Methods

An in-house quality standard was established in accordance with the *Vietnamese Pharmacopoeia V* [13], including the following evaluations:

2.2.1. Macroscopic description: Organoleptic evaluation was performed to describe the morphological characteristics and color of *M. pudica*.

2.2.2. Microscopic analysis: Transverse sections of leaves, stems, and roots were prepared. Observe them under a microscope (objective lens 40×, eyepiece 10×) and capture images of the anatomical structures of *M. pudica*.

2.2.3. Powder microscopy: Observe the powdered crude of *M. pudica* under a microscope using a 40× eyepiece and capture images of the diagnostic microscopic components present in the powder.

2.2.4. Qualitative identification by Thin-Layer Chromatography (TLC): TLC analysis was performed on silica gel GF254 plates using toluene – ethyl acetate (9 : 1, v/v) as the mobile phase. The test solution was prepared by extracting 1 g of powdered sample (355 µm) with 5 mL of methanol under sonication for 15 minutes, followed by filtration; the reference solution was prepared similarly using authenticated *M. pudica* powder. A volume of 20 µL of each solution was applied to the plate. After development, the plate was air-dried and examined under UV light at 254 and 366 nm, then sprayed with vanillin – sulfuric acid reagent, heated at 100°C, and observed under visible light. The chromatographic profiles of the test and reference solutions were compared based on spot color and R_f values.

2.2.5. Moisture content: Determined according to Appendix 9.6 of the *Vietnamese Pharmacopoeia V*. The test was performed on three batches.

2.2.6. Foreign matter: Determined according to Appendix 12.11 of the *Vietnamese Pharmacopoeia V*. Conducted on three batches.

2.2.7. Total ash: Determined according to Appendix 9.8 of the *Vietnamese Pharmacopoeia V*. Conducted on three batches.

2.2.8. Quantitative determination: Quantitative determination of mimosine was performed on three independent batches, each analyzed in triplicate, following a previously reported method [14]. The sample solvent used was 0.2 N hydrochloric acid (HCl). A stock standard solution (100 µg/mL) was prepared by accurately weighing approximately 2.5 mg of the mimosine reference standard into a 25 mL volumetric flask, dissolving it, and diluting to volume with the sample solvent. Working standard solutions (0.5 – 20 µg/mL) were subsequently prepared by appropriate dilution of the stock solution.

The test solution was prepared from dried herbal material that had been pulverized and passed through a 355 µm sieve, with loss on drying determined. An accurately weighed 400 mg portion of the powdered sample was moistened with approximately 1 mL of 50% ethanol, followed by extraction with 7 mL of 0.2 N HCl under sonication for 20 minutes at room temperature. The extract was decanted into a 20 mL volumetric flask, and the residue was re-extracted with 8 mL of 0.2 N HCl. The combined extracts were adjusted to volume with the same solvent and filtered through a 0.22 µm membrane before analysis.

Chromatographic separation was achieved on a Shim-pack GIST C18 column (250 × 4.6 mm, 5 µm) at 35°C

using a gradient elution system composed of sodium hexanesulfonate buffer (pH 2.0) and methanol. The flow rate was maintained at 1.0 mL/min, with a 20 µL injection volume. Detection was carried out at 275 nm, and the gradient conditions are summarized in Table 1.

Blank and standard solutions were injected to construct a calibration curve by plotting mimosine concentration against peak area. System suitability was considered acceptable when the correlation coefficient (R^2) was ≥ 0.998 (or ≥ 0.995). Based on the analytical results, an appropriate specification range for mimosine content in *Mimosa pudica* was proposed.

Table 1. *Gradient elution program for the quantitative analysis procedure*

Time (min)	Channel A (%)	Channel B (%)
0	90	10
10	90	10
11	75	25
20	75	25
21	90	10
25	90	10

Where: Channel A: Dissolve 4.0 g sodium hexanesulfonate in 800 mL distilled water, adjust pH to 2.0 with phosphoric acid (approximately 5 mL), and dilute to 1000 mL with distilled water. Channel B: Methanol.

The mimosine content (g/g) in the sample is calculated using the following equation: $H_{Mimosine}\ (\mu g/g) = \frac{C_x \times 20}{m}$

Where:

- C_x: concentration of mimosine in the test solution determined from the calibration curve (µg/mL)
- 20: dilution factor of the test solution
- m: mass of the test sample after moisture correction (g)

3. RESULTS AND DISCUSSION

3.1. Macroscopic characteristics

The stem bears recurved prickles. The leaves are bipinnately compound, with pinnae arranged in a digitate pattern. The main petiole is slender, approximately 4 cm in length, bearing two pairs of pinnae. Each pinna consists of 15 – 20 pairs of small, nearly sessile leaflets. The flowers are aggregated into ovoid heads. The pods are about 2 cm long and 3 mm wide, arranged in a star-like cluster, constricted between the seeds. The seeds are nearly oval, approximately 2 mm long and 1.5 mm wide.

3.2. Microscopic characteristics

3.2.1. Leaf anatomy

The transverse section of the leaf is illustrated in Figure 2 and described as follows:

Midrib: The cross-section is slightly convex on both adaxial and abaxial surfaces. The upper and lower epidermis consist of a single layer of polygonal cells with cellulose walls, irregular in size, and covered by a thin cuticle. Beneath the upper epidermis lies 1 – 2 layers of elongated palisade parenchyma cells. The spongy parenchyma comprises 1 – 2 layers of nearly uniform, rounded polygonal cells.

Vascular bundle: Arc-shaped and relatively narrow, with primary xylem located adaxially and primary phloem abaxially. The xylem consists of 5 – 6 rows of polygonal, lignified vessels, each row containing 2 – 3 vessels interspersed with 1 – 3 rows of xylem parenchyma cells with cellulose walls. The phloem is composed of polygonal cells with wavy cellulose walls, arranged in small clusters. The vascular bundle is surrounded (on the phloem side) by 2 – 3 layers of sclerenchymatous cells with thick, lignified walls. Prismatic calcium oxalate crystals are scattered in the parenchyma adjacent to the sclerenchyma.

Leaf lamina: The upper and lower epidermal cells are like those of the midrib, with a thin cuticle. Stomata are present on both surfaces (amphistomatic), with larger cells on the lower epidermis. Covering trichomes (1 – 2-celled) and glandular trichomes (with 1 – 2-celled stalks and multicellular heads of 1 – 4 cells) are sparsely distributed on the lower epidermis. The palisade parenchyma consists of 1 – 2 layers of elongated, closely packed cells. The spongy parenchyma occupies approximately two-thirds of the mesophyll, composed of irregularly arranged, rounded polygonal cells of varying sizes. Minor vascular bundles are scattered throughout the lamina, with a structure like the main vascular bundle but with fewer xylem and phloem elements; the phloem is subtended by a cluster of sclerenchymatous cells. Calcium oxalate crystals are also present in the mesophyll.

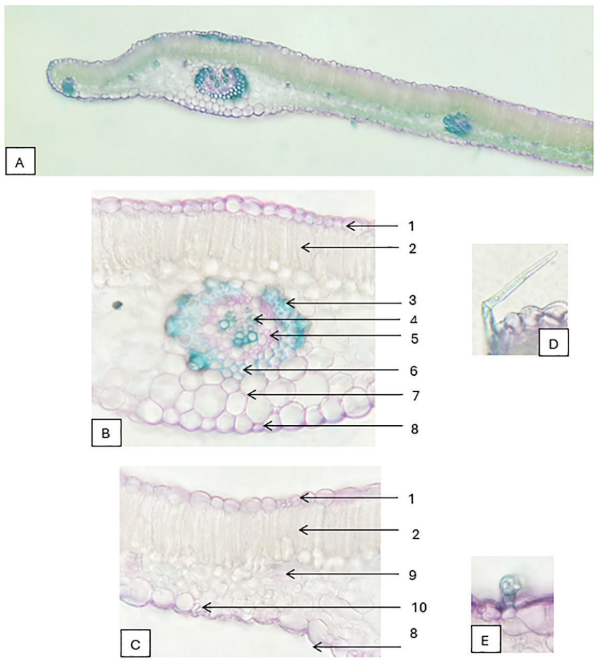


Figure 2. *Transverse section of the leaf of Mimosa pudica viewed under a microscope. (A) Whole transverse section; (B) enlarged view of the midrib region; (C) enlarged view of the leaf lamina showing: (1) upper epidermis, (2) palisade parenchyma, (3) prismatic calcium oxalate crystals, (4) primary xylem, (5) primary phloem, (6) sclerenchyma, (7) spongy parenchyma, (8) lower epidermis, (9) spongy mesophyll, and (10) stoma. (D) covering trichome; (E) glandular trichome.*

3.2.2. Stem anatomy

The transverse section of the stem (Figures 3 and 4) shows the following features:

The stem is circular in outline. The epidermis (1) consists of a single layer of cells, often partially sloughed off, with remnants of the cuticle and occasional protrusions corresponding to prickles. The cortex (2) is composed of oval parenchyma cells. Numerous groups of sclerenchymatous fibers (3) are arranged almost continuously in a ring. The secondary phloem (4) is wavy in outline. The vascular cambium (5) forms a continuous ring, producing secondary xylem (6) arranged concentrically, with localized protrusions where xylem vessels are more densely distributed. The pith (7) consists of parenchyma cells that are 2 – 4 times larger than those of the cortical parenchyma.

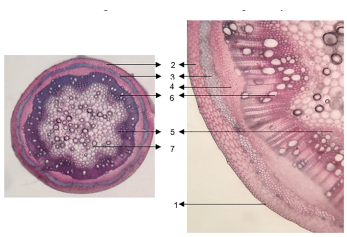


Figure 3. *Transverse section of the stem of Mimosa pudica.*

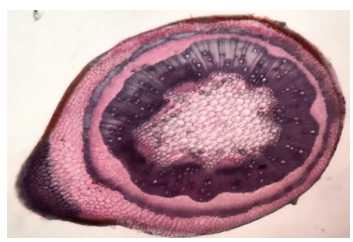


Figure 4. *Stem anatomy of Mimosa pudica showing remnants of protruding prickles.*

3.2.3. Root anatomy

The transverse section of the mature root shows the following features (Figure 5):

The mature root shows a cork layer (1) consisting of 5 – 12 tangentially arranged cell layers, with some of the outer layers being compressed or exfoliated. The cortical parenchyma (2) comprises 6 – 10 layers of thin-walled cells elongated in the tangential direction. The secondary phloem (3) includes sieve elements, fibers, crystal fibers, and phloem parenchyma, traversed by phloem rays. Phloem fibers occur singly or in groups, arranged in tangential bands. The secondary xylem (4) is composed of xylem elements intersected by medullary rays; vessels are scattered

throughout the xylem, with bordered pits and reticulate thickening of the walls. Thick-walled parenchyma cells are also distributed within the secondary xylem.

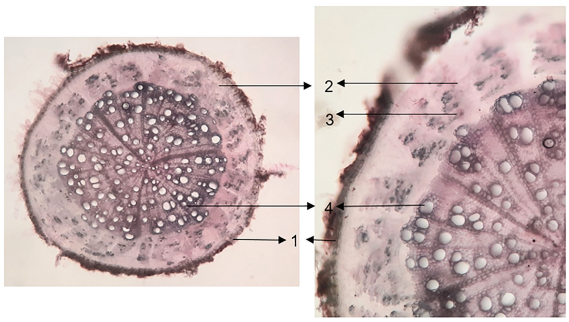
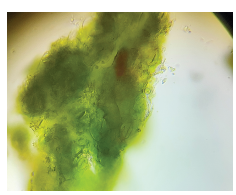


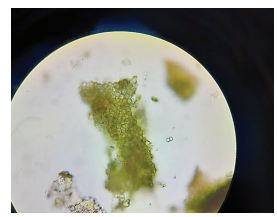
Figure 5. *Transverse section of the root of Mimosa pudica.*

3.3. Powder microscopy

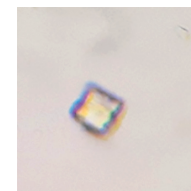
Powder microscopy of *M. pudica* under light microscopy revealed the following diagnostic features (Figure 6): Fragments of leaf epidermis with sinuous cell walls. Leaf mesophyll fragments contain abundant cytoplasmic content and green chloroplasts. Fragments of midrib and petiole parenchyma containing prismatic calcium oxalate crystals, usually isolated. Stem parenchyma fragments are composed of elongated cells containing starch granules; numerous starch granules are released, occurring singly, in pairs, or in groups of three. Vascular elements (vessel fragments). Rare fragments of fruit pericarp with irregularly angled cells containing small rhomboidal calcium oxalate crystals. Endosperm fragments are composed of cells lacking distinct angular shapes and filled with abundant cellular contents.



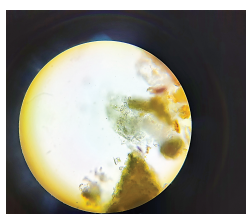
Leaf epidermal fragments



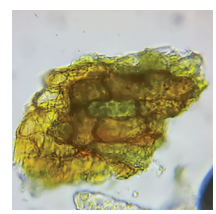
Leaf parenchyma fragments



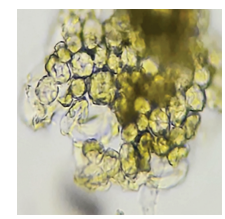
Calcium oxalate crystals



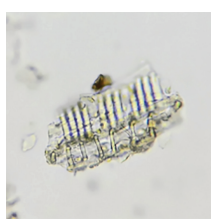
Stem parenchyma fragments composed of elongated cells containing starch granules



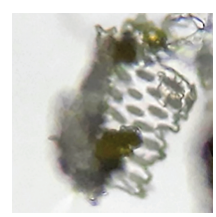
Stem parenchyma fragments composed of elongated cells



Endosperm fragments



Scalar vessels and spiral vessels



Reticulate vessels

Figure 6. *Microscopic characteristics of Mimosa pudica powder.*

3.4. Moisture content

The analysis of three batches of *Mimosa pudica* showed that the mean moisture content ranged from 9.54% to 10.66% (Table 2). Based on these results, the proposed acceptance criterion for moisture content, determined by loss on drying, is not more than 13%.

3.5. Foreign matter

The results from three batches indicated that the average foreign matter content ranged from 0.71% to 0.73% (Table 2). Accordingly, the proposed specification limit for foreign matter is not more than 1%.

3.6. Total ash

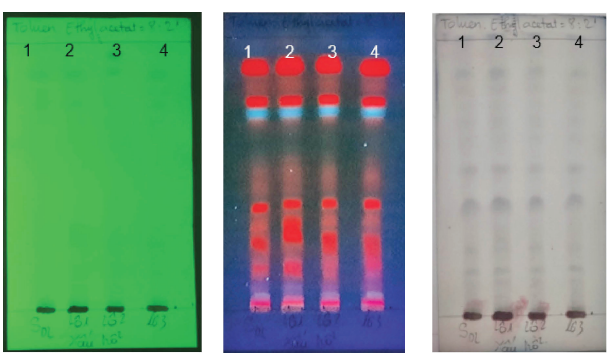
The total ash content determined from three batches ranged from 3.23% to 4.79% (Table 2). Based on these findings, the proposed limit for total ash is not more than 8%.

Table 2. *Analytical results of moisture content, foreign matter, and total ash in three batches of Mimosa pudica*

Batch	Moisture (%)	Foreign matter (%)	Total ash (%)
Batch 1	10.66 ± 0.05	0.73 ± 0.02	3.23 ± 0.13
Batch 2	9.54 ± 0.13	0.73 ± 0.03	3.74 ± 0.12
Batch 3	9.65 ± 0.47	0.71 ± 0.02	4.79 ± 0.03
Mean	9.95 ± 0.59	0.72 ± 0.02	3.92 ± 0.69
Specification	≤ 13%	≤ 1%	≤ 8%

3.7. Qualitative analysis (TLC)

Investigation of the mobile phase system for thin-layer chromatography indicated that the solvent system toluene - ethyl acetate (8 : 2, v/v) provided well-resolved spots. These spots were detectable under ultraviolet light at 254 nm and 366 nm and were further visualized after spraying with vanillin–sulfuric acid reagent. Evaluation of extraction solvents showed that the methanol extract exhibited the most diverse chemical profile (Figure 7).



UV 254 nm | UV 366 nm | Vanillin–sulfuric acid reagent

Figure 7. *TLC chromatograms for qualitative identification of three batches of Mimosa pudica: 1) Reference material, 2) Batch 1, 3) Batch 2, 4) Batch 3.*

3.8. Quantitative analysis

The determination of mimosine content was performed on three batches of *M. pudica*, with three replicate samples per batch (Table 3). The results showed that mimosine content ranged from 264.8 µg/g to 363.7 µg/g, calculated on a dry weight basis. Based on these findings, the proposed specification limit for the assay is not less than 160.0 µg/g, calculated on dried herbal material.

Table 3. *Mimosine content in three batches of Mimosa pudica*

Batch No.	Mean mimosine content (µg/g, n = 3)
1	363.7 ± 1.5
2	264.8 ± 0.6
3	± 0.8

3.9. Proposed in-house quality standard for *Mimosa pudica*

Based on experimental results and collected data, the proposed in-house specification is presented in Table 4.

Table 4. *Proposed in-house quality standard for Mimosa pudica*

No.	Parameter	Method	Specification
1	Macroscopic description	Organoleptic evaluation	Stem with recurved prickles. Leaves bipinnately compound with a digitate arrangement of pinnae; main petiole slender, ~4 cm long; two pairs of pinnae; leaflets 15 – 20 pairs, nearly sessile. Flowers in ovoid heads. Pods ~2 cm long, 3 mm wide, arranged in star-like clusters, constricted between seeds. Seeds nearly oval, ~2 mm × 1.5 mm.
2	Microscopy (anatomy)	According to the Vietnamese Pharmacopoeia V, Appendix 12.18	**Leaf:** Upper and lower epidermis like midrib, thin cuticle, stomata on both surfaces; lower epidermal cells larger. Covering trichomes (1 – 2 cells) and glandular trichomes (1–2-celled stalk, 1–4-celled head) are present. Palisade parenchyma 1 – 2 layers; spongy parenchyma about 2/3 of mesophyll. Minor vascular bundles are scattered, with sclerenchyma beneath phloem. Calcium oxalate crystals present. **Stem:** Circular cross-section; epidermis often partially lost; cortex of oval parenchyma cells; sclerenchyma fibers forming a near-continuous ring; secondary phloem wavy; vascular cambium and secondary xylem forming a ring; large pith cells. **Root:** Cork 5 – 12 layers; cortical parenchyma 6 – 10 layers; secondary phloem with sieve elements, fibers, and rays; secondary xylem with vessels having reticulate thickening; thick-walled parenchyma present.
3	Powder characteristics	According to the Vietnamese Pharmacopoeia V, Appendix 12.18	Leaf epidermal fragments with sinuous walls; mesophyll fragments rich in chloroplasts; parenchyma fragments with prismatic calcium oxalate crystals; elongated stem parenchyma with starch granules (single or grouped); vessel fragments; rare pericarp fragments with rhomboidal crystals; endosperm fragments with abundant contents.
4	Identification	Thin-layer chromatography (TLC)	Under UV light at 366 nm and after derivatization with vanillin–sulfuric acid (VS) reagent, the chromatogram of the test solution must show spots with the same color and R_f values as those of the reference solution.
5	Loss on drying	Vietnamese Pharmacopoeia V, Appendix 9.6	Not more than 13.0%
6	Foreign matter	Vietnamese Pharmacopoeia V, Appendix 12.11	Not more than 1.0%
7	Total ash	Vietnamese Pharmacopoeia V, Appendix 9.8	Not more than 8.0%
8	Assay (mimosine)	HPLC-PDA	Not less than 160.0 µg/g (calculated on dried material)

4. CONCLUSION

An in-house quality standard for *Mimosa pudica* has been successfully established, including the following parameters: macroscopic description, microscopic characteristics (plant anatomy), moisture content (loss on drying), total ash, foreign matter, qualitative identification by thin-layer chromatography (TLC), and quantitative determination of mimosine using high-performance liquid chromatography (HPLC).

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