

## ANATOMICAL STUDIES ON THE *MUCUNA* SPECIES NATIVE TO TAIWAN

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*Mucunae Caulis* was used as “Xueteng” in herbal medicine in Taiwan. This study identified and compared the internal structures of the plant stems of the genus *Mucuna* ADANSON in the Leguminosae family native to Taiwan. The dried stems were microsectioned in transverse and longitudinal direction for the microscopic studies. Micromorphological studies showed *mucunae* species that had reddish rings with stone cells and lignified fibers at the cortex layers outer. In the inner cortex layer, *M. macrocarpa* and *M. pruriens* var. *utilis* had no scattering lignified cells. *M. gigantea* had lignified cells sporadically congregated and the lignified cells of *M. membranacea* were spread vertically and continuously. Using this method, the original sources of *Mucuna Caulis* native in Taiwan could be compared and identified precisely.

**Key words:** *Mucuna*, Xueteng, Gixueteng, Anatomy.

### INTRODUCTION

The genus *Mucuna* ADANSON (Leguminosae) comprises of about 160 species distributed over the area of tropics and subtropics<sup>1</sup>. *Mucunae Caulis* were used as “Xueteng”<sup>2</sup>, in folk medicine for numbness, paralysis, pain around the waist and knee, and menstruation malfunction<sup>2,3</sup>. Additionally, some people used the stem of *Mucunae Caulis* as “Gixueteng”, which could be obtained from many other plants sources<sup>3,4</sup>. The *Mucuna* species is largely distributed all over the Taiwan along the side paths in the valleys and also at low altitude forests. According to our investigation, the *Mucuna* plants grown in Taiwan consist of three species and one variety, namely: *M. gigantea*, *M. macrocarpa*, *M. membranacea*, *M. pruriens* var. *utilis*<sup>5-7</sup>. The present study was carried out to identify the histological characteristics of the stems in different species of *Mucuna*.

## MATERIALS AND METHODS

### Plant materials

1. The *M. gigantea* were collected from the mountains of Hentzun County, Taiwan in March, 1999.
2. The *M. macrocarpa* were collected from the North-Cross Highway, Taiwan in July, 1999.
3. The *M. membranacea* were collected from Lanyu, Taiwan in April, 2000.
4. The *M. pruriens* var. *utilis* were collected from Yutzih, Taiwan in July, 2000.

All the plants were identified by Mr. Nien-Yung Chiu, technician, Institute of Chinese Pharmaceutical Sciences, China Medical University.

### Experimental methods

The internal structure

1. Cross section: The free-hand cross sections of herbal stems were made by using sharp knife followed by cleaning with 5 % chloral hydrate solution and examined under the microscope. The slices were managed and further observed for special tissues by the following methods.
  - (1) Add Sudan III solution followed by glycerin-alcohol-water (1:1:1) to examine the cork layer.
  - (2) Add one drop of concentrated 3 % phloroglucinol-alcohol solution followed by one drop of 1N hydrochloric acid to exhibit the lignified cells.
  - (3) Add one drop concentrated sulfuric acid followed by one drop of  $\alpha$ -naphthol to test the inulin.
2. The vertical and surface cutting segments of stem were part in the test tube, concentrated nitric acid and small amount of potassium chlorate was added the test tube was heated to decompose the tissue.

## RESULTS AND DISCUSSION

(1) The external morphology of *Mucuna* plants was similar to that described in Flora of Taiwan<sup>3</sup> and listed it in Table 1.

(2) Histological characteristics

(I) *M. gigantea*

Magnifying glasses were used to examine the cross section of the stem, the outermost layers were the dark brownish cork layers, the cortex was thin and had a purplish red colored ring nearly in the outer edge, the vascular bundle occupied more than one-third of the cross sections area, and the pith occupied three-quarters in the center (Fig. 1B).

**Table 1. Comparison of the external shapes of *Mucunae* plant**

Plant Characteristics	<i>M. gigantea</i> (Fig. 1A)	<i>M. macrocarpa</i> (Fig. 2A)	<i>M. membranacea</i> (Fig. 3A)	<i>M. pruriens</i> var. <i>utilis</i> (Fig. 4A)
Small branches	Glabrous	Rusted brown pubescent hair	Glabrous	Young branches covered with brown hairs
Leaves	Semi-leathery, glabrous terminal leaflet in long elliptical shape, cuspidate	Semi-leathery, with rushed brown backs, terminal leaflet in long elliptical shape, cuspidate	Leaflet membranaceous in ovate-rhomboid shape, terminal leaflet obtuse, short cuspidate and acute base, with thick hairs on both sides	Leaflet membranaceous in ovate long elliptical shape, with acute top and short cuspidate, base in wedge shape, glabrous on the surface and covered with hairs underneath
Length of leaves (cm)	12-15	12-15	10-12	8-12
Racemes	Long peduncle, pendulous	Racemes long peduncle, pendulous	Flowers in axillary racemes	pendulous racemes
Calyx	With a few bristles, the margin almost truncate yellowish green corolla	Without rusted-colored hair dark purple corolla	Large flower in dark purple or deep purple colors, with long pubescent hairs and 5 teeth	Flower in purple, with long hairs underneath the style
Pods	L: 8-14 cm, 4-5 cm, densely covered with bristles with 2-6 seeds	Flattened, L: 20-40 cm W: 5cm, densely covered with short pubescent hairs with 4-12 seeds	Broad elliptical shape, 10-15cm long, with many protrude lines	Flattened, 4-10 cm long, densely covered with white hairs with vertical ribs, 2-5 seeds
Place of production	Southern Taiwan	places in Taiwan at low and middle altitudes	Lanyu, Lutau	Southern Taiwan

The transverse section of the stem was shown in Fig. 1C. The outermost edge of stem was 1-2 layers of epidermis which was oblong, flattened oblong or square-like shape. The outer layer came with brownish yellow cuticular cells.

The inner side of the epidermis was 2-8 layers of cork cells. The cells showed oblong or square shape and 13-22  $\mu\text{m}$  in diameter (Fig. 1D<sub>1</sub>).

The cortex layer (Fig. 1D<sub>1</sub>), occupied about one-third to a half of the section area was composed of 12-20 layers of oblong or square shape parenchyma which was about 8-192  $\mu\text{m}$  in diameter and contained innumerable starch

grains, calcium oxalate crystals and yellowish white secretion substances. The starch grains showed simple and round in shape with 0.3-0.5  $\mu\text{m}$  in diameter (Fig. 1D<sub>2</sub>). The calcium oxalate crystals were 0.7-1.9  $\mu\text{m}$  in diameter (Fig. 1D<sub>3</sub>).

The reddish ring near the outer edge of the cortex consisted of 2-16 layers of stone cells and lignified fibers (Fig. 1D<sub>4</sub>). The stone cells manifested thick walls, strongly lignified, round-like, long elliptical or square in shape and a size of 4-87  $\mu\text{m}$ , as well as conspicuous striation and pits.

Cambium was composed of 5-7 layers of cells in flattened square or tangential shape.

Xylem was circularly lined, wider than the phloem and took up about one-third of section area. Xylem fibers, wood parenchymas and 8-33 layers of medullary rays were present. The vessels had bordered pit, reticulate, scalariform, and spiral form, the diameter ranged 150-350  $\mu\text{m}$  and the length ranged 160-635  $\mu\text{m}$  or even longer (Fig. 1D<sub>5</sub>). The xylem fiber cells were square, ellipse, and polygon in shape (Fig. 1D<sub>6</sub>) and 120-887  $\mu\text{m}$  or even longer in length and were lignified or softly lignified.

The pith cells close to the center were larger than that in periphery. These cells were rather thick walls, long-ellipse-like, and polygon-like in shape.

#### (II) *M. macrocarpa*

Magnifying glasses were used to examine the cross section of the stem; the outermost layer was brownish and grayish cork layer. The cortex occupied about one-fourth of the section area and was comprised of dark purple-purplish red ring nearly at the outer edge, the vascular bundle occupied more than a half of the section area and pith was in center (Fig. 2B).

Fig. 2C shows the internal structures characteristics as observed in the transverse sections of the stem. The outermost edge was 1-2 layers of epidermis showed oblong, flattened form and yellowish brown in color.

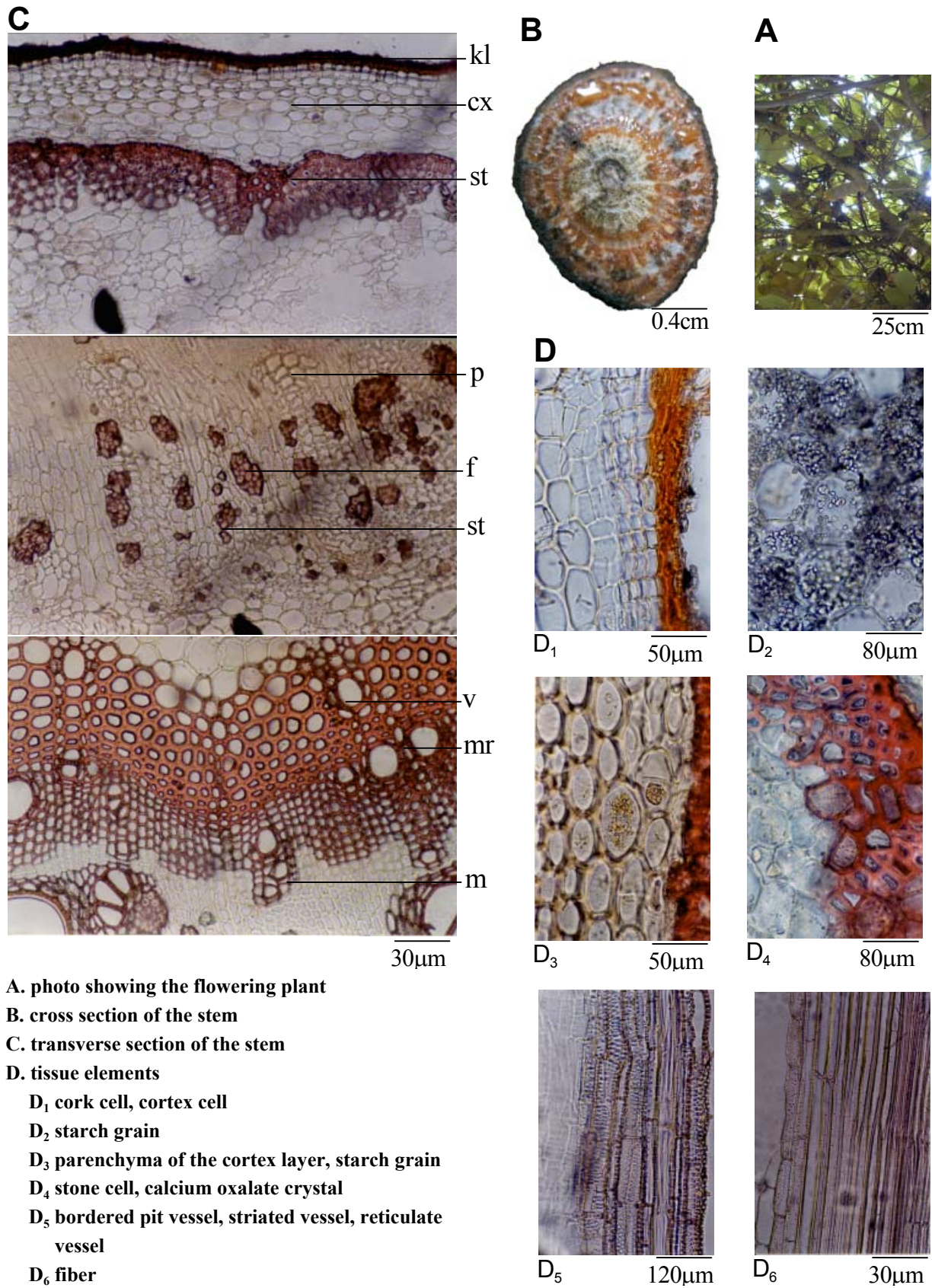
Cork comprised of 4-22 layers, the cells showed oblong, oblong-like or square-like shape, the diameter was 25-43  $\mu\text{m}$  (Fig. 2D<sub>1</sub>).

Cortex layer took up about one-fourth to one-third of the section area, was composed of 9-34 layers of oblong, round, ellipse parenchyma which was 7-135  $\mu\text{m}$  in diameter and contained innumerable starch grains, calcium oxalate crystals and yellow or yellowish white secretions substances (Fig. 2D<sub>2</sub>). The starch grains were individual or cluster with 0.3-1.2  $\mu\text{m}$  in diameter. The calcium oxalate crystals were 0.6-1.4  $\mu\text{m}$  in diameter.

The dark-reddish ring near the outer cortex layer outer consisted of 4-12 layers stone cells and lignified fibers. The stone cells (Fig. 2D<sub>3</sub>) showed thick walls, strongly lignified, round, ellipse, long elliptical, or square in shape and conspicuous striation and pith and were about 12-145  $\mu\text{m}$  in diameter.

Cambium cells comprised of 4-7 layers flattened square cells were tangentially spreaded.

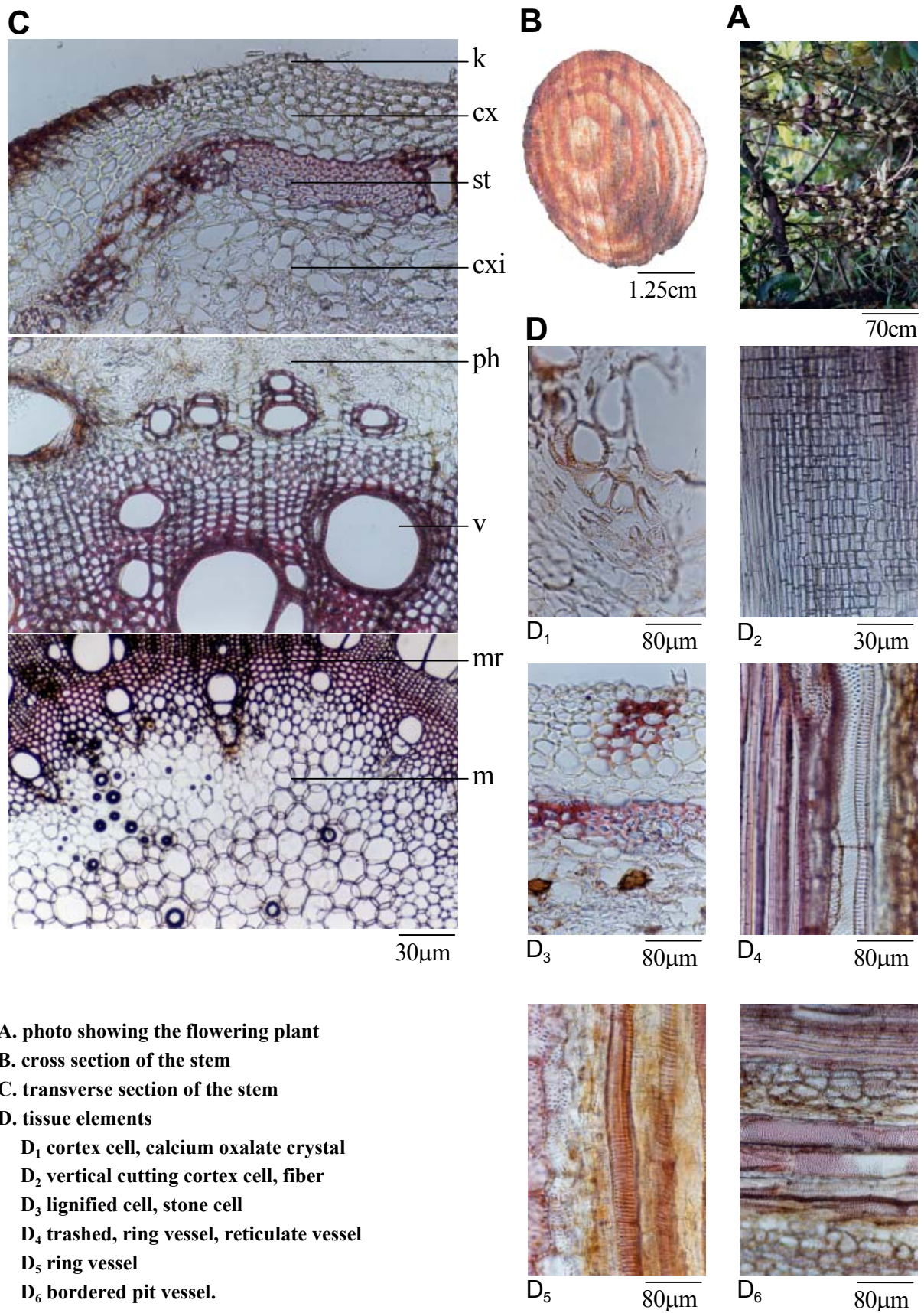
Xylem was wider than phloem and was arranged circularly. It composed of xylem fibers, and wood parenchyma. The xylem fiber, parenchyma and vessels were grown alternatively. The vessels had reticulate vessels, ring vessels and bordered pit vessels (Fig. 2D<sub>4</sub>, 2D<sub>5</sub> and 2D<sub>6</sub>), the diameter ranged 130-630  $\mu\text{m}$  and the length was 145-776  $\mu\text{m}$  or even



**A.** photo showing the flowering plant  
**B.** cross section of the stem  
**C.** transverse section of the stem  
**D.** tissue elements  
 D<sub>1</sub> cork cell, cortex cell  
 D<sub>2</sub> starch grain  
 D<sub>3</sub> parenchyma of the cortex layer, starch grain  
 D<sub>4</sub> stone cell, calcium oxalate crystal  
 D<sub>5</sub> bordered pit vessel, striated vessel, reticulate vessel  
 D<sub>6</sub> fiber

**Fig. 1.** *Mucuna gigantea* (WILLD.) DC.





**Fig. 2. *Mucuna macrocarpa* WALL.**

longer. The medullary rays were extended laterally with 7-25 layers. The xylem fiber cells were square, round and polygon in shape and the length was 97-1045  $\mu\text{m}$  or even longer.

Pith cell walls were slightly thick, round or ellipse in shape. The pith cells close to the center were larger in size than those in the periphery.

(III) *M. membranacea*

Magnifying glasses were used to examine the cross section of the stems. The outermost layer of the stem was the brownish gray cork, followed by the cortex layer which occupied half of the section area. The lignified cells occupied one-third of the cross section area. Pith was in the center (Fig. 3B).

The Internal structures characteristics of transverse section of the stem is shown in Fig. 3C.

The outermost layer of the stem comprised of 1-2 layers of epidermis which showed yellowish brown in color and oblong, flattened oblong or square-like in shape, followed by 2-5 layers of cork layer which were oblong or square shape with 25-70  $\mu\text{m}$  in diameter (Fig. 3D<sub>1</sub>).

Cortex layer occupied one-third to one-fourth of the section area, was composed of 21-41 layers ellipse, round, oblong or square shape parenchyma with 16-66  $\mu\text{m}$  in diameter (Fig. 3D<sub>1</sub>). Starch grains showed simple and round with 0.5-2.3  $\mu\text{m}$  in diameter. In addition, there were some 0.3-0.5  $\mu\text{m}$  of calcium oxalate crystals and yellowish white secretions substances presented (Fig. 3D<sub>3</sub>).

The reddish ring near the outer of the cortex layer consisted of 4-6 layers stone cells and lignified fibers. These stone cells manifested thick walls, strongly lignified, round, ellipse, long elliptical or square in shape with approximately 2-20  $\mu\text{m}$  in diameter, conspicuous striation and pits (Fig. 3D<sub>2</sub>).

Cambium comprised of 4-7 layers of cells in flattened square shape and were extended tangentially.

Xylem was wider than the phloem and occupied one-third of the section area. Xylem fiber, xylem parenchyma and medullary rays were also present. The strongly lignified vessels had bordered pit, reticulate and ring in shape (Fig. 3D<sub>4</sub> and 3D<sub>5</sub>), the diameter was of 25-450  $\mu\text{m}$ , and the length was of 12-680  $\mu\text{m}$  or even longer. The medullary rays had 16-28 layers and were extended vertically. The lignified or slightly lignified xylem fibers appeared square-like, round-like, and polygon-like in shape, the length was of 85-520  $\mu\text{m}$  or even longer. The crystal fibers were also present (Fig. 3D<sub>6</sub>).

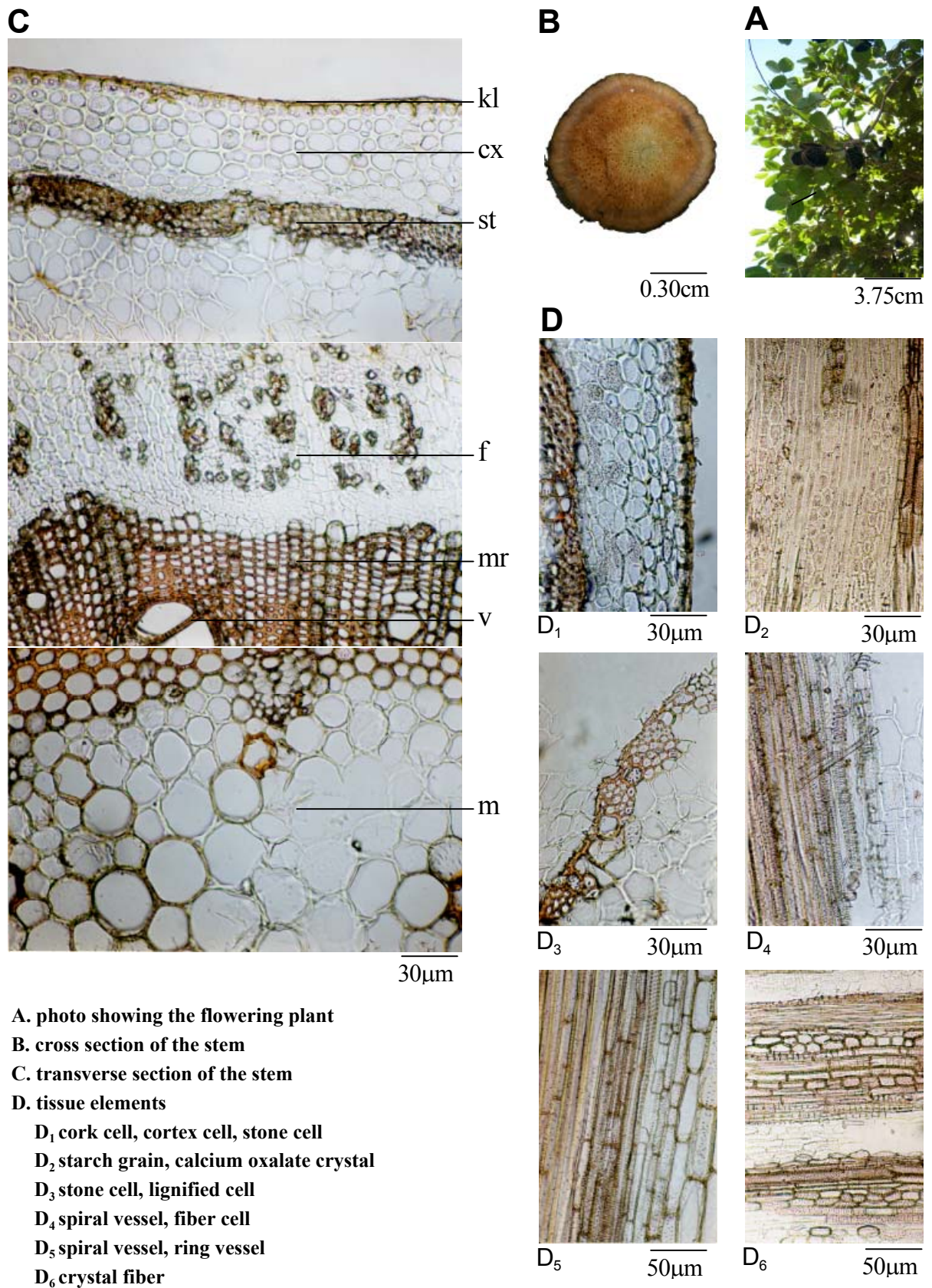
Pith cells were thick walls, round, long ellipse and polygon in shape. The size of pith cells closed to the center with larger than that of periphery.

(IV) *M. pruriens* var. *utilis*

Magnifying glasses were used to examine the cross sections of the stems, the outermost layer was the dark brownish gray cork layer, followed the cortex layer which occupied more than one-fifth of the section area. The lignified cells occupied one-fourth of the section area, and broadly pith was found in the center (Fig. 4B).

The internal structures characteristics of the transverse section of the stem were shown in Fig. 4C. The outermost





**Fig. 3.** *Mucuna membranacea* HAYATA



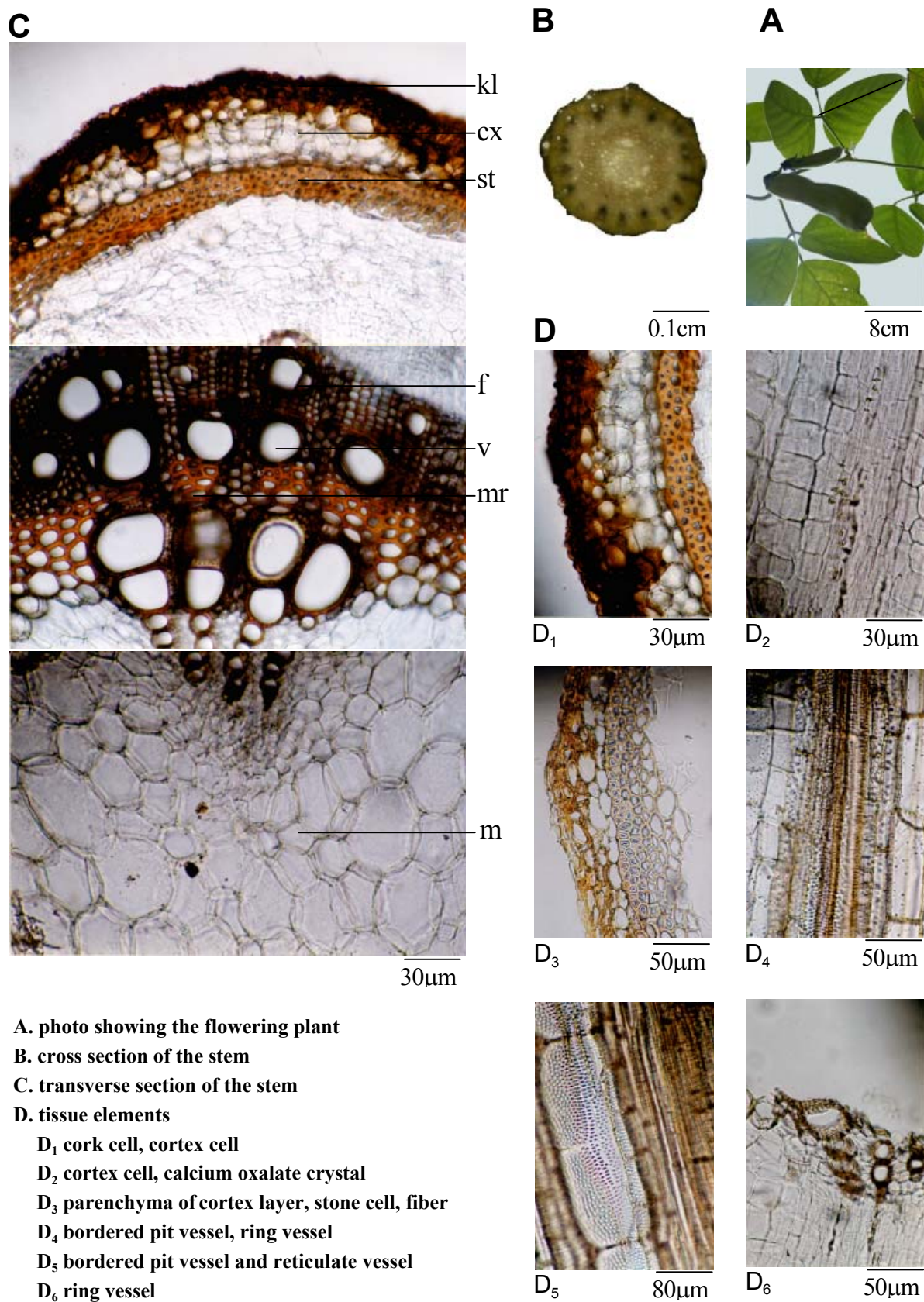


Fig. 4. *Mucuna pruriens* (L.) DC. var. *utilis* WALL. ex WIGHT.

**Table 2. The comparison of the internal structures of *Mucunae* Caulis**

Microscopic characteristics	<i>M. gigantea</i>	<i>M. macrocarpa</i>	<i>M. membranacea</i>	<i>M. pruriens</i> var. <i>utilis</i>
Cork layer	2-8 layers 13-22 $\mu\text{m}$	4-22 layers 25-43 $\mu\text{m}$	2-5 layers 25-70 $\mu\text{m}$	4-6 layers 8-41 $\mu\text{m}$
Cortex layer	12-20 layer 8-192 $\mu\text{m}$	9-34 layers 7-135 $\mu\text{m}$	21-41 layers 16-66 $\mu\text{m}$	11-21 layers 20-58 $\mu\text{m}$
Stone cells	2-16 layers 4-87 $\mu\text{m}$	4-12 layers 12-145 $\mu\text{m}$	4-6 layers 2-20 $\mu\text{m}$	3-8 layers 4-54 $\mu\text{m}$
Cambium	5-7 layers	4-7 layers	4-7 layers	3-5 layers
Fiber cell	120-887 $\mu\text{m}$	97-1045 $\mu\text{m}$	85-520 $\mu\text{m}$	120-460 $\mu\text{m}$
Vessel cell	Bordered pit, Reticulate, scalariform, spiral	Bordered pit, Reticulate, spiral	Bordered pit, spiral, ring	Bordered pit reticulate, ring
Diameter:	150-350 $\mu\text{m}$	130-630 $\mu\text{m}$	25 - 450 $\mu\text{m}$	16-355 $\mu\text{m}$
Length:	160-635 $\mu\text{m}$ or even longer	145-776 $\mu\text{m}$ or even long	12-680 $\mu\text{m}$ longer or even longer	150-360 $\mu\text{m}$ or even longer
Medullary ray	8-33 layer	7-25 layer	16-28 layer	7-18 layer
Crystals	0.7-1.9 $\mu\text{m}$	0.6-1.4 $\mu\text{m}$	0.3-0.5 $\mu\text{m}$	0.3-1.5 $\mu\text{m}$
Starch grain	0.3-0.5 $\mu\text{m}$	0.3-1.2 $\mu\text{m}$	0.5-2.3 $\mu\text{m}$	0.3-1.1 $\mu\text{m}$
Crystal fiber	—	—	+	+

was made of one layer oblong, flattened square or square epidermis cells.

Cork comprised of 4-6 layers oblong, square-like or oblong-like shape cells, and about 8-41  $\mu\text{m}$  in diameter.

Cortex layer occupied one-third to a half of the section area and consisted of 11-21 layers round, ellipse, or oblong parenchyma cells with 8-37  $\mu\text{m}$  in diameter (Fig. 4D<sub>1</sub>). Starch grains were simple or round and with 0.3-1.1  $\mu\text{m}$  in diameter. Besides, there were also some 0.3-1.5  $\mu\text{m}$  of calcium oxalate crystals and yellow or yellowish white secretion substances presented (Fig. 4D<sub>2</sub>).

The reddish rings were made of 3-8 layers stone cells and lignified fibers in the middle near the outer of the cortex layer. These stone cells were thick wall, strongly lignified, round, ellipse, long elliptical, or square in shape, conspicuous striation, pith and were about 4-54  $\mu\text{m}$  in diameter (Fig. 4D<sub>3</sub>).

Cambium was made of 3-5 layers of cells in flattened square shape and was extended tangentially.

The xylem was arranged circularly, wider than the phloem and occupied one-third of the section area. Xylem fibers, xylem parenchyma, vessels and medullary rays were also present. These cells were lignified and grown alternately. The shape of vessels was reticulate, ring, and bordered pit, the diameter was 16-335  $\mu\text{m}$  and the length was

150-360  $\mu\text{m}$  or even longer. The medullary rays comprised of 7-18 layers and extended vertically. The lignified xylem fibers appeared in square, round, and polygon in shape and were 120-460  $\mu\text{m}$  or even longer in length (Fig. 4D<sub>4</sub>, 4D<sub>5</sub> and 4D<sub>6</sub>).

Pith cells were round, ellipse, long ellipse, and polygon in shape with thick walls. The pith cells closed to the center were larger than that of the periphery.

The comparison of the internal structures of *Mucunae* spp. was listed it on Table 2. All *Mucuna* species had a reddish ring consisted of stone cells and lignified fibers near the outer cortex layer. In the parenchyma region at the inside cortex, *M. gigantea* and *M. membranacea* had strongly lignified cells scattered, the former showed more regularly and sporadically, but the latter was spread out vertically and continuously. The ratio of the cortex layers to lignified cells, *M. macrocarpa* was from one-third to a half of the section area, *M. pruriens* var. *utilis* was 1:1. Both of them had no other scattering lignified cells at the cortex parenchyma region.

The species of *Mucuna* plants used as the sources of “Gixueteng” in Taiwan were: *M. gigantean* and *M. macrocarpa* in Taiwan. Using this method, we could precisely compare and identify the source of all “*Mucunae* Caulis”.

It is feasible to distinguish the stem of three species and one variety of genus *mucuna* based on their morphological and histological characteristics such as presence or absence of crystals and oil droplets in stem, thickness of cuticle, and distribution of sclerenchymatous cells etc.

This study has established the identification characteristics of *Mucuna* spp. by making comparison of the external morphology and the internal characteristics of *Mucuna* stem. These crude drugs of Xeuteng Caulis need to be sliced, in order to precisely identify the genetic origin of Formosan *Mucunae* Caulis from others. Identifying the genetic origin makes the herbal choice more correct, also ensures the dose regimen exactly and increases the safety medicine usage.

**List of abbreviations:** bs, vascular bundle; c, cambium; co, collenchyma; cu, cuticular; cx, cortex; cxi, inner cortex; ep, epidermis; f, fiber; kl, cork layer; kc, cork cambium (phellogen); mr, medulla ray; m, pith; p, parenchyma; sec, secretory cell; st, stone cell; sta, starch; vc, scalariform vessel; vd, bordered pit vessel; vg, ring vessel; vp, pitted vessel; vr, reticulate vessel; vs, spiral vessel; wf, wood fiber; xy, xylem.

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# 台灣產血藤屬植物之組織學研究

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血藤屬 (*Mucuna*) 植物可用於飲片藥材，一般稱為血藤。本研究辨別並比較不同種之台灣產豆科血藤屬植物藤莖的內部構造。將乾燥藤莖經顯微橫切及縱切後、製成切片以顯微鏡觀察研究。研究顯示本屬所有植物於皮層有石細胞及木化纖維之紅色環。血藤 (*M. macrocarpa*) 和刀豆 (*M. pruriens* var. *utilis*) 於內皮層無散生木化細胞。恆春血藤 (*M. gigantean*) 有木化細胞非連續性聚生，蘭嶼血藤 (*M. membranacea*) 內皮層之木化細胞呈連續性、垂直性散生延伸。本研究可精確比較和確定台灣血藤屬 (*Mucuna*) 中藥材種類的原始基原。

**關鍵詞：**血藤屬，血藤，雞血藤，解剖學。