Morphological and Histological Characterization of *Ichnocarpus frutescens* R.Br. (Twinnet-kado)

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Abstract

The medicinal plant Ichnocarpus frutescens R.Br. is commonly known as Twinnet-kado, belonging to the family Apocynaceae were collected from Pathein University Campus, Pathein Township. The collected plants were classified and identified with the help of literatures for morphological and microscopical characters. In the morphological study, the plant was a woody twiner; leaves simple, opposite and decussate; inflorescens terminal and axillary paniculate cymes; flowers actinomorphic, greenish-white, hypogynous; carpels 2, parietal placentation, disk erect linear lobes; seeds linear. In microscopical study, the leaves are dorsiventral. The stomata type is paracytic were located on upper and lower surfaces. Calcium oxalate prisms are scattered in the parenchymatous cells. The stems are circular in outline, the epidermal cells one-layered thick, the cortex is made up of collenchymatous cells towards the peripheral region and parenchymatous cells towards the inner region. Unicellular, uniseriate trichomes are present. T.S of young root showing air cavities, unicellular, uniseriate trichomes present, xylem polyarch. In old root, the cortex layers have no cavities, made up of parenchymatous cells.

Key words: Ichnocarpus frutescens R.Br., medicinal plant, morphological, microscopical, parenchymatous cell.

INTRODUCTION

Myanmar is floristically rich in medicinal plants and supporting 85% of the population in rural areas. Most of the people use traditional medicine for the treatment of diseases. Although synthetic drugs and antibiotic brought about a revolution in controlling different diseases, plants occupy a very significant role as raw material for some important drugs [21].

So in this research, one traditional medicinal plant was selected. Namely *Ichnocarpus frutescens* R.Br. belongs to the family Apocynaceae and is also known as Twinnet-kado, Taw-sabe in Myanmar and Black creeper in English [7], [11].

Ichnocarpus frutescens R.Br. is a woody twiner, which grows wild in the leaf-shedding forests, especially in the mixed ones and the savannahs, all over Myanmar; Bago, Sagaing, Shan, Taninthayi, Yangon and Pathein Area [11].

The root is sweetish, cooling, aphrodisiac, thirst, vomiting, fever, biliousness, diseases of the blood. The root is considered to pass alterative tonic properties. The stalks and leaves are used in the form of decoction in fevers [10].

The fresh vegetative and floral parts were studied for morphological and histological characters of their leaves, stems and roots.

The aim and objectives of the present study are to identity and verify the morphological and histological characters of various plant parts; to study the structural characteristics of medicinal parts.

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MATERIALS AND METHODS

Collection and Identification of *Ichnocarpus frutescens* R.Br.

The specimens in this study were collected from Pathein University Campus during September, 2010 to February 2013.

After collected specimens were measured, recorded in detailed for taxonomic description, then identified in the Department of Botany, Pathein University with the help of literatures [2], [3], [4], [6], [10], [16], [17], [20].

The habit, inflorescences, leaves and flowers of the specimen collected were recorded by using digital camera. Herbarium specimens were also prepared by using collected specimens according to the method which was mentioned in Herbarium techniques [14].

Histological study of *Ichnocarpus frutescens* R.Br.

Numerical values such as stomatal index, palisade ratio, veins islet and veins termination number in leaves [5], [13], [15], [18], [19].

In histological studies of lamina, midribs, petioles, stems and roots were examined by preparing free hand sections from the fresh specimens.

The free hand sections and powdered samples were examined by the reagents are chloral hydrate solution BP for clearing reagent, 1% phloroglucinol BP followed by concentrative hydrochloric acid for lignin, acetic acid and concentrative sulphuric acid for calcium oxalate crystals, N/50 iodine solution for starch grains and tannin substances were tested by 3% ferric chloride solution [18], [19].

Preparation of powdered samples

The collected samples (leaves and roots) were washed with water. After washing the samples were air dried and ground to get powdered and stored in air tight containers.

Determination of Stomatal Number and Stomatal Index

$$S.I = \frac{S}{E+S} \times 100$$

S.I = stomatal index

S = number of stomata per unit area

E = number of ordinary epidermal cells in the same unit area [18]

Determination of Palisade ratio

Pieces of leaf about 2 mm square or powder are cleared by boiling with chloral hydrate solution, mounted and examined with a 4 mm objective. The palisade cells lying beneath each epidermal cell are focused and traced. The palisade cells in each group are counted, the figure obtained divided by 4, gives the palisade ratio of the group.

RESULTS

Morphological Characters of *Ichnocarpus frutescens* R.Br.

A woody twiner up to 2 m in height; stems much branched, brownish, glabrous, sap milky. Leaves simple, opposite and decussate, exstipulate, petiolate, brownish green, pubescent; blades elliptic-oblong, the bases rounded to cuneate, the margins entire, the tips acuminate, the upper surface green and glabrous, the lower pale green and slightly pubescent. Inflorescences terminal and axillary paniculate cymes, peduncle long and slender, slightly pubescent. Flowers actinomorphic, greenish - white, small, fragrant, bracts - leafy, the bases cuneate, the tip - acuminate, bracteolate, pedicellate, bisexual, regular, 5 - merous, hypogynous. Sepals 5, basally connate, ovate - acute, minute, green with brown hairs, without glands inside, persistant. Corolla salver - shaped, widened above the base, slightly pubescent, mouth

villous, the lobes 5, white, upper half deflexed in bud, pubescent, overlapping to the right, the lobes undulate at the margin, glabrous on the outer side, hairy within. Stamens 5, inserted at the middle of the corolla tube, petalostemonous, the filament very short, the anther sagittate, acute, short apical appendages, conniving over and adhering to the stigma, dithecous, longitudinally dehiscent. Ovaries oblongoid, hairy, carpels 2, distinct, ovules many, parietal placentation; the style single, stout, the stigmas ovoid - columnar, disk 5 - lobed, erect linear lobes. Fruits follicles very slender, cylindric, rusty- pubescent at first, afterwards glabrous. Seeds linear, black. Flowering and fruiting period is from September to February. Found growing wild in Pathein University Campus. The results were shown in Figures 1-9.

Morphological Characters of *Ichnocarpus frutescens* R.Br.

Fig. 1. Habit

Fig. 2. Inflorescences

Fig. 3. Leaves

Fig. 4. Close up view of a flower Fig. 5. L.S of flower

Fig. 6. Androecium Fig 7.. T.S of ovary Fig. 8. Follicles Fig. 9. T.S of fruit

Microscopical Characters of *Ichnocarpus frutescens* R.Br.

Lamina

In surface view, upper epidermal cells are rectangular to polygonal in shape and anticlinal wall straight. Lower epidermal cells are polygonal, anticlinal wall slightly wavy. Stomata of upper surfaces are absent. Stomata of lower epidermal cells are abundant, paracytic type. The stomata are oval in outline with two reniform - shaped guard cells. Uniseriate, unicellular trichomes are present on upper surface.

In transverse section, the cuticle are thin, present on both surfaces. The upper epidermal cells are rectangular in shape, more larger than the lower epidermal cells, thin-walled, compactly arranged. Under the upper epidermal cells, the hypodermis are one-layered, parenchymatous. Palisade cells found below the hypodermis are small and arranged at right angle to the surface and one-layered thick. The spongy mesophyll cells are 5- to 6- layers of irregular-shaped, thin-walled, parenchymatous cells with large intercellular spaces. Calcium oxalate crystals (prisms) are present in mesophyll cells. These characters are the same in apical regions, central regions and basal regions but different in sizes.

The vascular bundles are collateral type and different in sizes according to apical, central and basal regions. Xylem lied towards the upper epidermis and composed of vessels, tracheids, fibres-tracheids, fibres and xylem parenchyma. Phloem lied towards the lower epidermis and consisted of sieve tubes, companion cells and phloem parenchyma cells. The results were shown in Figures 10-12.

Histological Characters of *Ichnocarpus frutescens* R.Br.

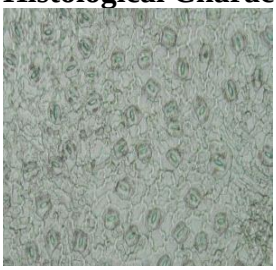

Fig. 10. Surface view of Upper epidermis

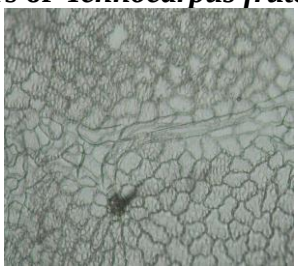

Fig. 11. Surface view of Lower epidermis

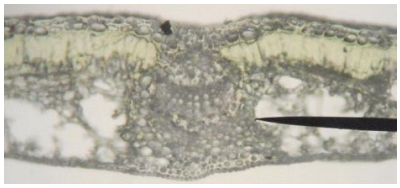

Fig. 12. T.S of Lamina

Midribs

In surface view, the upper epidermal cells are thin - walled, rectangular in shape, unicellular, uniseriate, trichomes are present. The lower epidermal cells are smaller than the upper ones and calcium oxalate crystals (prisms) are present. The cells are elongated along the length of the midrib.

In transverse section of midrib the cuticle is thin in apical, central and basal regions. The upper epidermal cells are parenchymatous, compactly arranged. The lower epidermal cells are smaller than the upper epidermis. Below the upper epidermis, the collenchyma are 2- to 3-layers. The cells are isodiametric and rounded in shape. The number of these cell layers are different in apical, central and basal regions. Parenchyma cells above the vascular bundle are 10- to 15-layers (apical), 18- to 22-layers (central), 22- to 25-layers (basal) and below the vascular bundle are 7- to 8-layers (apical), 10- to 13-layers (central), 12- to 14-layers (basal). The cells are thin - walled, irregular-shaped, compactly arranged. Calcium oxalate crystals (prisms) are present in the parenchymatous cells. Trichomes are present on the upper surface.

The vascular bundle of midribs are collateral type, numbers of medullary rays reducing towards apex. Xylem composed of vessels, tracheids, fibres- tracheids, fibres and xylem parenchyma cells. In apical, central and basal vascular bundle, protoxylem lied towards the upper surface and metaxylem towards the lower side. Phloem cells are thin-walled, consisted of sieve tubes and companion cells and phloem parenchyma cells. The results were shown in Figures 13-16.

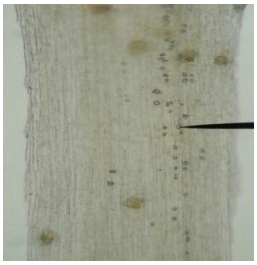

Fig. 13. Upper surface of Midrib

Fig. 14. Lower surface of Midrib

Fig. 15. T.S of Midrib

Fig. 16. Close up view of of Midrib

Petioles

In surface view, the epidermal cells are thin-walled and rectangular in shape, unicellular, uniseriate trichomes are present.

In transverse section, the cuticle is thin, the epidermal cells are parenchymatous, compactly arranged, unicellular, uniseriate trichomes are present on the upper epidermis. Below the epidermis, the outer and inner collenchyma cells have 4- to 5-layers on both sides. The parenchymatous cells are polygonal to rounded, 22- to 25- layers of parenchyma cells above the vascular bundle and 8- to 10-layers below and calcium oxalate prisms are scattered in the parenchymatous cells. Trichomes are present on the upper surface.

The vascular bundles are crescent-shaped, collateral type, medullary rays present, xylem lied towards the upper epidermis and compose of vessels, tracheids, fibres-tracheids, fibres and xylem parenchyma and phloem lied towards the lower epidermis and composed of sieve tubes, companion cells and phloem parenchyma cells. The results were shown in Figures 17-19.

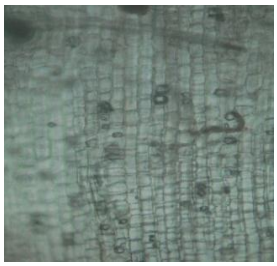

Fig. 17. Upper surface of Petiole

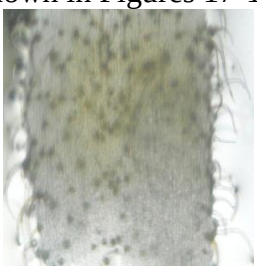

Fig. 18. Lower surface of Petiole

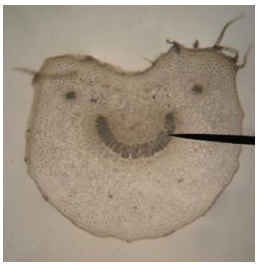

Fig. 19. T.S of Petiole

Stems

In surface view, epidermal cells are elongated, simple and warty trichomes are present.

In transverse section, the young and old stems are circular in outline. The epidermal cells are rectangular in shape. Under the epidermis, the hypodermis is one-layered, rectangular in shape. Cortex is made up of 4- to 6 - layers of collenchyma cells, towards the peripheral region. 5- to 7 - layers of parenchymatous cells towards the inner region. Phloems are 4- to 5 - layers, present outside the vascular strands. Xylems are composed of annular, spiral vessels, fibres, fibres-tracheids, tracheids, and xylem parenchyma. Phloems are composed of sieve tubes and companion cells, phloem fibres and phloem parenchyma. The central pith is parenchymatous, 30- to 40-layers, intercellular spaces in old stem are larger than the young stem. Abundant tannin content, starch grains are found in the parenchyma cells. Trichomes in young stems are larger than the old ones. The results were shown in Figures 20-21.

Fig. 20. T.S of Young Stem with warty trichomes

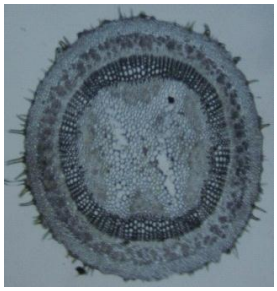

Fig. 21. T.S of old stem with warty trichomes

Roots

In surface view, epidermal cells were parenchymatous, thin-walled, rectangular in shape, the cells were elongated along the length of the root.

In transverse section of young root was circular in outline. The epiblema cells were single layered and rectangular in shape. They are parenchymatous and root hairs are present. Below the epiblema, hypodermis one-layered and rectangular in shape. The cortex lied below the hypodermis layer, 5- to 6 - layers of parenchymatous cells, rounded to polygonal, intercellular spaces are present. The endodermis composed of single layered of barrel-shaped, parenchymatous cells surrounding the vascular bundle. Pericycle one - layered, parenchymatous cells, rectangular in shape. Pith cells were parenchymatous, oval to rounded in shape. Xylem polyarch, composed of vessels, fibres, fibres-tracheids and tracheids. Phloem composed of sieve tube and companion cell and phloem parenchyma cell.

The old root is circular in outline, phellem or cork one - layered and thin - walled, rectangular in shape, the phellogen or cork cambium 2- to 3 - layers, thin-walled cells and rectangular in shape. The phelloderm or secondary cortex lies next to the phellogen. It consisted of 3- to 5 - layers, rectangular to polygonal in shape parenchymatons cells. The cortical region consists of 10- to 16 - layers, rounded to polygonal in shape, parenchymatous cells. Medullary rays are present at the centre around the pith. Tannins were present in the cortical region. The results were shown in Figures 22-23.

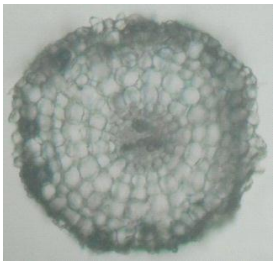

Fig. 22. T.S of young root

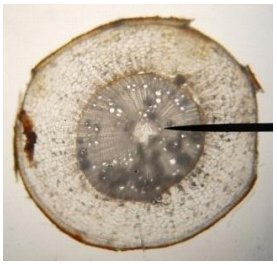

Fig. 23. T.S of old root

Diagnostic characters of powdered leaves and roots

Leaves-greenish, roots greenish-brown; round to polygonal-shaped, parenchymatous cells, pitted vessels, tracheids, fibres-tracheids, unicellular, uniseriate trichomes and paracytic stomata.The results were shown in Figures 24-29 and Tables 1,2.

Table 1 Trichomes and crystals characters of *Ichnocarpus frutescens* R.Br.

Plant part use	Trichomes	Crystals
Leaves and roots	Unicellular and Uniseriate	Prismatic

Diagnostic Characters of Powdered Leaves and Roots of *Ichnocarpus frutescens* R.Br

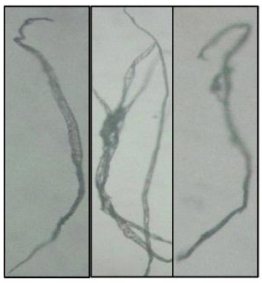

Fig. 24. Fibres

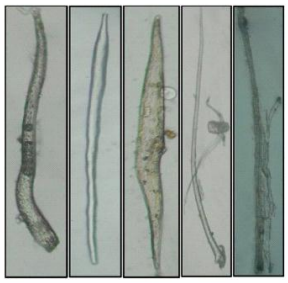

Fig. 25. Tracheids

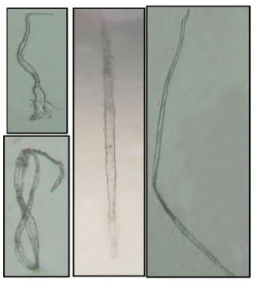

Fig.26. Fibre-Tracheids

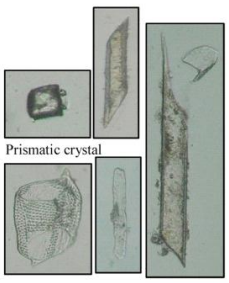

Fig. 27. Pitted-vessel elements

Table 2 Sensory Characters of the Powdered Leaves and Roots

Sensory characters	Leaves	Roots
Colour	Greenish	Greenish-brown
Odour	Slightly Aromatic	Aromatic
Taste	Slightly bitter	Sweetish
Texture	Granular, fibres	Granular, fibres

Powdered Leaves and Roots of *Ichnocarpus frutescens* R.Br.

Figure 1.1 Leaves

Figure 1.2 Roots

DISCUSSION AND CONCLUSION

One traditional medicinal plant, *Ichnocarpus frutescens* R.Br. belongs to the family Apocynaceae and is also known as Twinnet-kado, Taw-sabe in Myanmar and Black creeper in English [7], [11].

Ichnocarpus frutescens R.Br. is a woody twiner, which grows wild in the leaf shedding forests, especially in the mixed ones and the savannahs, all over Myanmar; Bago, Sagaing, Shan, Taninthayi, Yangon and Pathein Area [11].

The plant of *Ichnocarpus frutescens* R.Br. is a woody twiner, the pubescent hairs on the leaves, stems and branches, elliptic oblong leaves, many flowers condensed on paniculate cymes, corolla tube with salver-shaped, villous mouth, disk present. The above characters are in accordance with [1], [2], [3], [4], [6], [10], [12], [16], [17].

In histological studies, the leaves are dorsiventral, the epidermal cells are rectangular to polygonal in shape, anticlinal walls slightly wavy. The stomata type is paracytic. In transverse section of midrib, the vascular bundles are collateral. Calcium oxalate prisms are scattered in the parenchymatous cells of cortical regions. In T.S of petiole, the vascular bundles are arc-shaped collateral, uniseriate trichomes are present on the upper epidermis. In T.S of young stem and old stem are circular in outline, epidermal cells are barrel-shaped, one-layered thick and the cortex is made up of collechymatous cells towards the peripheral region and parenchymatous cells towards the inner region. In T.S of young root, the epiblema cells are single-layered, parenchymatous cells. Young root showing air cavities. Unicellular, uniseriate trichomes are present. The cortex are parenchymatous, xylem polyarch. In the old root, phellem or cork one-layered, phellem or cork cambium 2 - to 3 - layers, phelloderm or secondary cortex are 3 - to 5 - layers. The cortical region consists of 10 - to 16 - layers, parenchymatous cells. Periderm is less prominent. Tannins are present in the cortical regions.

In sensory characters, leaves-greenish, roots greenish-brown; round to polygonal-shaped parenchymatous cells, pitted vessels, tracheids, fibres-tracheids, unicellular, uniseriate trichomes and paracytic stomata were observed.

The above characters are in agreement with those given by [8], [9], [13].

The method of identification of characteristics and histology of medicinal parts is established and the results provide a basis for identification of medicinal materials. Extensive research is fundamentally important to control the quality of raw drugs and the formulation to justify their uses in the modern medicine system.

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