

## MORPHOLOGICAL AND HISTOLOGICAL STUDIES OF *GNETUM GNEMON* L. (TANYIN-YWE) FROM YE-PHYU TOWNSHIP

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### Abstract

The *Gnetum gnemon* L. is an indigenous plant from Thaninthayi Region. The specimens were collected from Ye-Phyu Township, during July 2018 to January 2019. Its aerial parts such as leaves, strobili and seeds are used by local people for food. The main purpose of this study was to examine the morphological and histology of the leaves, stems, roots and seeds of *Gnetum gnemon* L. The morphological features were described. The specimens were evergreen dioecious small trees, the leaves were simple, opposite, lanceolate to elliptic, and exstipulate. The male strobilus has 5-9 involucre collars, each collar contains 3-6 male flowers, stamen 1, adnate to the base of perianth, anther bifid. The female strobilus was 5-9 collars, each collars with 3-6 female flowers, without perianth, orthotropous ovule, pendulous placentation. The seeds were large and drupe. The distinguished histological characters of *Gnetum gnemon* L. revealed paracytic type of stomata in lower surface view of lamina only, pitted non lignified tracheary elements occurred in both surface view of lamina, cortex region of petiole, midrib and stem as well as in roots. Asterosclereids are commonly found in the whole plant parts except T.S of lamina. The vascular bundles in T.S of stem are collateral and close type. In addition, starch containing parenchymatous cells is found only in phelloderm region of mature roots. Moreover, the seed coat comprises sarcotesta, sclerotesta and inner endotesta regions. Underneath the seed coat is the endosperm containing starch grains and proteinaceous globules.

**Keywords:** strobilus, orthotropous ovule, asterosclereids, sarcotesta, *Gnetum gnemon* L.

### Introduction

*Gnetum* is the sole genus within the family Gnetaceae and the order Gnetales of the gymnosperm. This genus consists of about 40 species and widely distributed in South America, West Africa, tropical and subtropical Asia (Fell *et al.*, 2015). According to Kress *et al.*, 2003, there are 7 species of genus *Gnetum* were found in Kachin State, Taninthayi and Sagaing Regions in Myanmar. The selected sample, *Gnetum gnemon* L. belongs to the family Gnetaceae. It is known as Hyinbyin, Tanyin-ywe in Myanmar, “Melinjo” in Indonesia and Japan, Bago in Phillipines, Mejhergut in India. Local people from Ye-Phyu Township has been used the leaves and seeds of *G. gnemon* L. as vegetable crop. *G. gnemon* L. is a dioecious evergreen tree. The trunk is smooth and cylindrical, the branches are opposite and symmetrical branches. Leaves are simple and opposite, elliptic, lanceolate or oblong-oval. As a gymnosperm, *Gnetum gnemon* L. does not have true flower; the cones or strobili are presented at the tip of a slender stem or axis. It has yellow single-seeded fruits (Fell *et al.*, 2015).

*Gnetum gnemon* L. widely cultivated in Southeast Asia. The fruit, seed, leaves and flowers of this plant can be consumed. This plant is the stable food in some places. *Gnetum gnemon* L. rich in resveratrol. The plant of *Gnetum gnemon* L. is used to reduce the level of sugar in the blood, anti-inflammatory activity and induce apoptosis in colon cancer (Hafidz *et al.*, 2017). The seed extract of *Gnetum gnemon* L. is sold as a nutritional supplement in Japan (Narayanan *et al.*, 2015). It is reported that the leaves of *Gnetum gnemon* L. contain bioactive compounds such as saponins, stilbenoids, isovitexin and gnetins. However, the scientific investigations on *Gnetum gnemon* L. are still lacking in Myanmar. Therefore, the aim of the present study is to identify the morphological characters of *Gnetum gnemon* L. and to investigate the histological

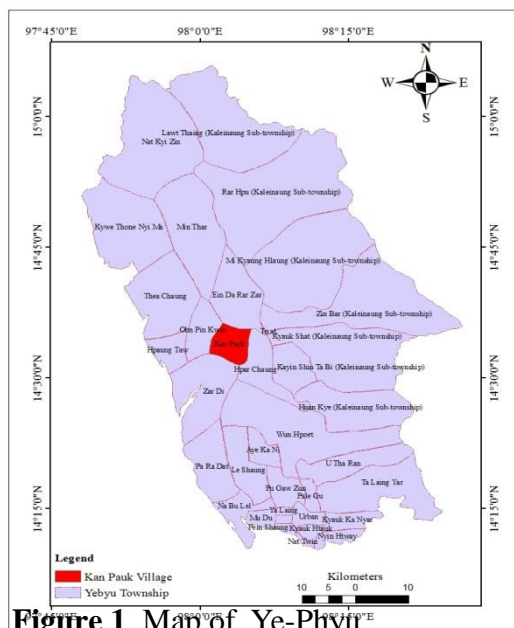
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studies of leaves, stems, roots and seeds of *Gnetum gnemon* L. from Ye-phyu Township, Taninthayi Region.

### Materials and Methods

#### I. Morphological study of *Gnetum gnemon* L.



#### Collection, Classification and Identification

The plants use in this research were collected from Ye-Phyu Township (North Latitudes 14° 35' 49.8" and East Latitude 98° 02' 14.4"), Taninthayi Region during July 2018 to January 2019. Fresh specimens were used for classification and identification with the help of standard literatures. Collected specimens were examined and described according to standard method used in Department of Botany, University of Yangon.

14° 35' 49.8" N  
98° 02' 14.4" E

Figure 1 Map of Ye-Phyu

#### Characteristics of Gymnosperm (Flora of Hong Kong, Vol I., 2007)

Trees or shrubs, rarely woody vines: mostly evergreen: vascular bundles arranged in circles, with cambium, secondary xylem consists of tracheids, rarely with vessels. Leaves usually linear, needle-shaped or scale-like. Cones generally unisexual, mostly dioecious, globose, conical to cylindrical; ovules naked, not enclosed in an ovary. Seeds with endosperm, embryo straight: cotyledon 2 to many.

#### Key to the Families (Flora of Hong Kong, Vol I., 2007)

- 1a. Leaves large, partitioned, fascicled on top of stem.
  - 2a. Pinnae with midrib..... Cycadaceae
  - 2b. Pinnae without midrib, with many longitudinal veins..... Zamiaceae
- 1b. Leaves small, not partitioned.
  - 3a. Leaves fan-shaped, veins dichotomous..... Ginkgoaceae
  - 3b. Leaves not fan-shaped, veins not dichotomous.
    - 4a. Woody vines: leaves simple, opposite, veins reticulated: flowers with pseudoperianth..... Gnetaceae
    - 4b. Trees.

**Key based on male plant (Flora of China, Vol II., 1995)**

- 1a. Shrubs or small trees; leaves drying yellowish; spikes often very lax visible between involucre collars.....*G. gnemon*
- 1b. Vines; leaves drying dark green to brown or black; spikes always very dense with axis concealed by involucre collars and flowers.

**Key based on female plant (Flora of China, Vol II., 1995)**

- 1a. Shrubs or small trees; leaves drying yellowish; seed coat usually velvety, rarely glabrous.....*G.gnemon*
- 1b. Vines; leaves drying dark green to brown or black; seed coat glabrous or with minute, silvery scales.

**II. Histological study of *Gnetum gnemon* L.**

Microscopical study of leaves, stems, roots and seeds were also examined by using free hand sections. The free hand sections were cleared in chloral hydrate solution on a glass slide, stained with safranin and temporary mounted by glycerin and then observed under a light microscope (Esau, 1964; Biswas and Johri, 1997 and Vasishta *et al.*, 1939).

**Determination of Stomatal index**

The average number of stomata per square millimeter of epidermis is termed the stomatal number. The percentage proportion of the ultimate divisions of the epidermis of a leaf, which have been converted into stomata, is termed the stomatal index (Trease and Evans, 1978).

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

S.I = stomatal index

S = number of stomata per unit area

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

**Determination of palisade ratio**

The average number of palisade cells beneath each epidermal cell is termed the 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. First a number of each group of four epidermal cells is traced and their outlines inked in to make them more conspicuous. The palisade cells lying beneath each epidermal cell are focused and traced. The palisade cells in each group are counted, those being included in the count which are more than half-covered by the epidermal cells, the figure obtained divided by 4, gives the palisade ratio of the group (Trease and Evans, 1978).

**Results**

**I. Morphological characters of *Gnetum gnemon* L.**

Scientific Name - *Gnetum gnemon* L.

Local Name - Hyinbyin, Tanyin-ywe

English Name - Melinjo, belinjo, bago, maninjau, voe, khalel, peedae, phak, gam cay, bet, mejherguti, letera

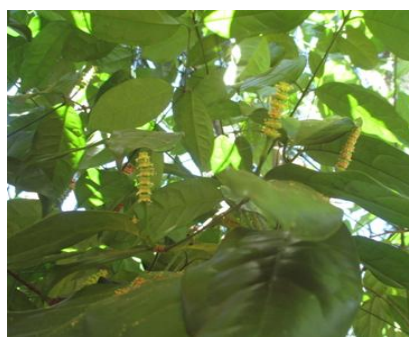
Family - Gnetaceae

Flowering and Fruiting period - October to February

Evergreen dioecious small trees (2-30 m) in high; bark grayish (Fig.2 and Fig.9); branches opposite and decussate, green or yellowish green. Leaves simple, opposite, 10cm-22cm long, 3cm-7cm wide, petiole 0.8cm-1.5cm, lanceolate in young leaves and elliptic in mature leaves, 8.3cm-14.8cm long, 2.7cm-5cm wide, the tips acuminate, the margins entire, the bases acute, reticulate venation, exstipulate (Fig. 4-5) and (Fig.10-11). Male spikes axillary or terminal, 4.5cm-7.3cm (Fig.6), involucre collars clearly separated, one male spike contain 5-9 involucre collars, to 1cm apart, each collar contains 3-6 male flowers and a single ring of 3-9 globose sterile female flowers (Fig.8), male flowers with a claw-shaped, 0.2cm-0.3cm, perianth narrowly clavate, 0.1cm, entire, stamen one, stamen adnate to the base of the perianth, 0.1cm, synangia (anther) bifid (Fig.7). Female spikes axillary or terminal, 4.5cm-9.0cm (Fig. 10), one female spike contains 5-9 collars, to 1 cm apart, each collars with 3-9 female flowers (Fig. 13), female flower are globose (Fig.14), without perianth, each containing of a single orthotropous ovule, with three integuments, the inner one produced into a fimbriate mouth, placentation pendulous (Fig.15-16). Seed large, drupe-like, ellipsoid, 1.5cm-1.8cm, longitudinally ribbed (Fig.20), green in young (Fig.17) and yellowish-red when mature (Fig. 1), consisting of the fleshy coats, enclosing the hard seed. External seed coat green, thick, internal seed coat brown, thin, ovule large and white.



**Figure 2** A male plant in nature habit



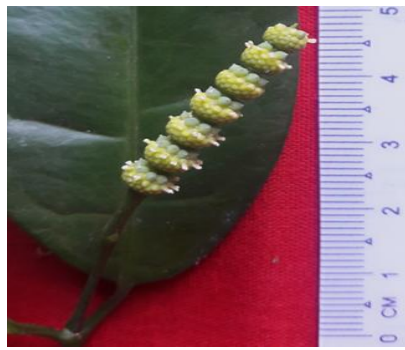
**Figure 3** Male strobilus with nature plant



**Figure 4** Upper surface of



**Figure 5** Lower surface of leaves



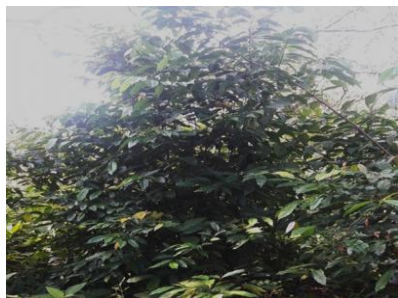
**Figure 6** Male strobilus



**Figure 7** Male flowers



**Figure 8** Globose abortive ovules



**Figure 9** A female plant in nature habit



**Figure 10** Female strobilus with nature plant



**Figure 11** Upper surface of leaves



**Figure 12** Lower surface of leaves



**Figure 15** T.S of ovary



**Figure 16** L.S of ovary



**Figure 17** Cluster of seeds



**Figure 18** Cluster of ripen seeds



**Figure 19** Ripen seeds



**Figure 20** Dry seeds

## II. Histological characters of *Gnetum gnemon* L.

### Lamina

In surface view of lamina, paracytic type of stomata are present only in lower surface (Fig. 22). The subsidiary cells in lower surface views are waiver than the upper surfaces. The stomatal index is 15.0-18.5 and palisade ratio is 3.0-10.5. The vascular bundles in T.S of lamina is collateral and closed type (Fig.23). In T.S of lamina, the upper and lower epidermal cells are covered by thick cuticle, a single layer of palisade mesophyll cells lie beneath the upper epidermal cells, 6-8 layers of spongy mesophyll cells lie internal to the palisade layer, the cells are thin wall and loosely arranged with intercellular spaces. Pitted non lignified tracheary elements are scattered in mesophyll layers.

### Midribs

In Transverse section, the midrib is slightly convex in adaxial region and concave in abaxial region, a single layer of epidermis is covered by cuticle. The cortex is composed of 10-13 layers of parenchymatous cells in adaxial side and the cells are more or less rounded, where as 16-19 layers in abaxial side (Fig. 24). There are 4-5 bundles lie in the center of midrib and the arrangement of bundle is collateral and close type (Fig.25).

### Petioles

The petiole of *Gnetum gnemon* L. is slightly flattened with two wings at the upper region and semicircular-shaped in lower region a single layer of epidermis is covered by cuticle, the cortex is made up of 15-18 layers of parenchymatous cells in adaxial region and 15-20 layers in abaxial region. The arrangement of vascular bundles are crescent- shaped, xylem lying inward and

the phloem lying outward. Astrosclereids are found in hypodermis regions of midribs as well as petioles (Figure 26).

### Stems

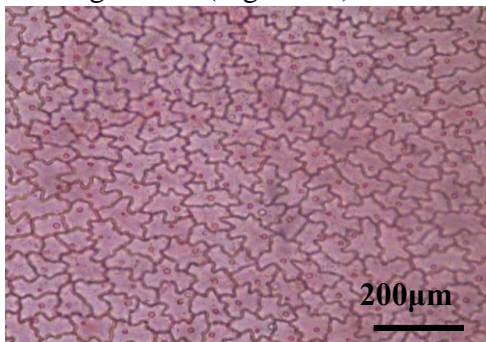
In transverse section, the stem, is cylindrical-shaped, the cortex region is composed of outer collenchymatous, middle parenchymatous and inner sclerenchymatous region. The pitted non lignified tracheary elements scattered in cortex region of stem. The vascular bundles are collateral and closed type. The xylem is composed of tracheids and vessels whereas phloem is made up of sieve tube and phloem parenchyma cells (Fig.27).

### Roots

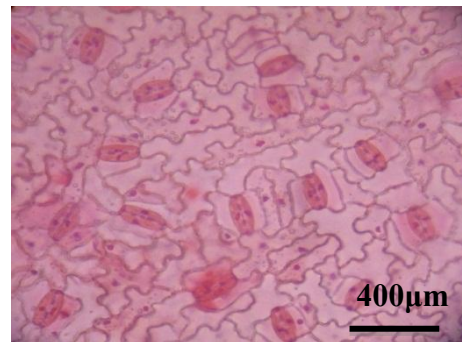
In transverse region of root, the periderm region is composed of phellogen or cork cambium, phellem or cork cells and phelloderm. The circular pitted non lignified tracheary elements are scattered in phelloderm region of root. The endodermis is not distinct. Vascular bundles occur as protostele type and concentric type (Fig. 28).

### Seeds

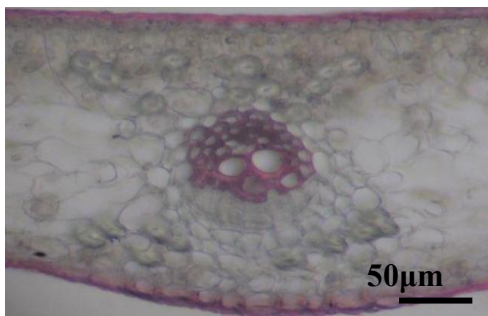
In T.S of seeds, the seed coat is made up of outermost sarcotesta, middle sclerotesta and inner endotesta. The sarcotesta has cutinized epidermis and group of asterosclereids region (Fig. 29). The sclerotesta forms the protective layer of the seed. It is made up of upper sclerenchymatous region and inner membranous region (Fig.30). The endotesta is composed of more or less rounded parenchymatous cell and small vascular bundles are also occur in this region. The endosperm comprises parenchymatous cells filled with starch grains and proteinaceous granules (Fig.31-32).



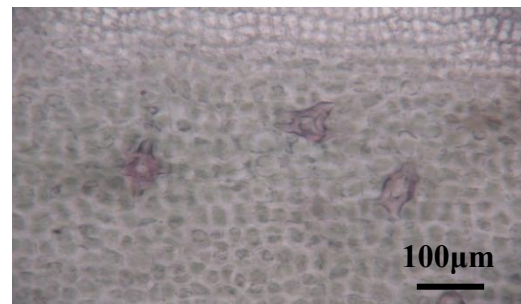
**Figure 21** Upper surface view of lamina



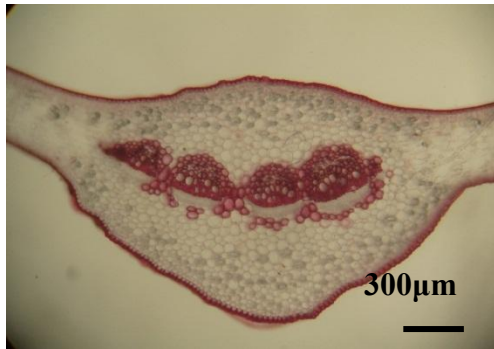
**Figure 22** Lower surface view of lamina



**Figure 23** Close up view of lamina showing vascular bundle



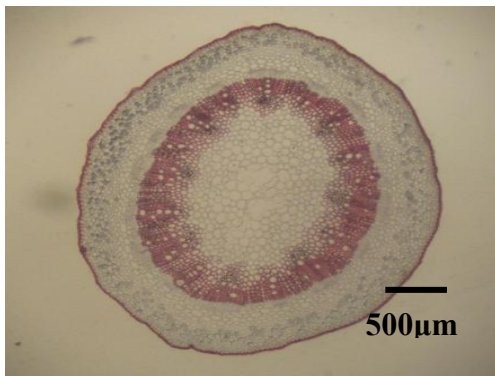
**Figure 24** Astrosclereids are found in surface view of midrib



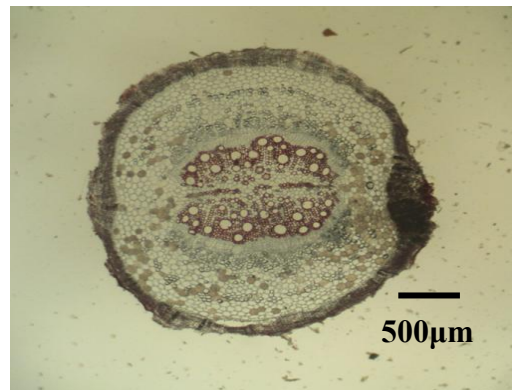
**Figure 25** T.S of midrib



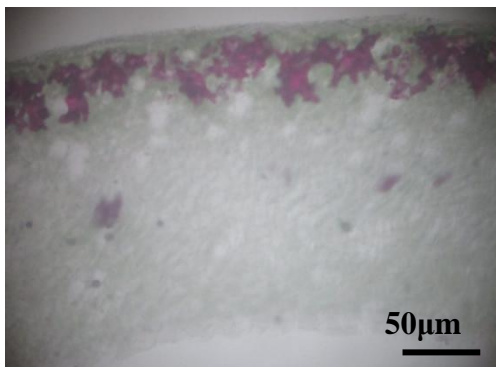
**Figure 26** T.S of petiole



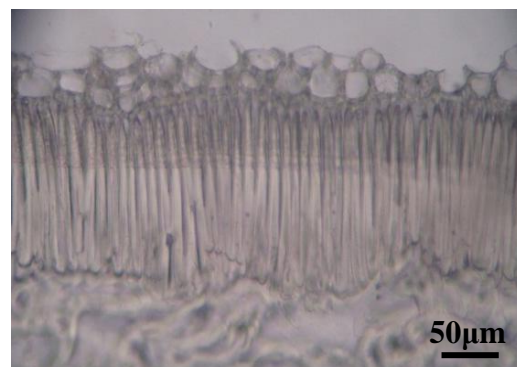
**Figure 27** T.S of stem outline



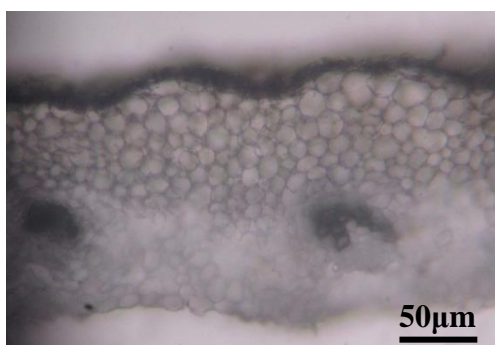
**Figure 28** T.S of root outline



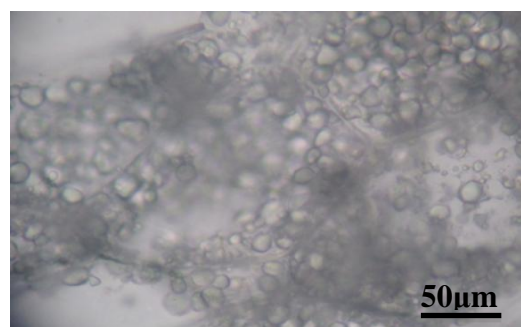
**Figure 29** T.S of seed showing Asterosclereids



**Figure 30** T.S of seed coat showing middle sclerotesta



**Figure 31** T.S of seeds showing endotesta



**Figure 32** Starch grains and proteinaceous globules found endosperm region



## Discussion and Conclusion

In the present research, the morphological characters on the vegetative parts, reproductive parts and histological characters of leaves, stems, roots and seeds have been undertaken. Thus, the specimen used in this research was identified as *Gnetum gnemon* L. and it belongs to the family Gnetaceae.

*Gnetum gnemon* L., *G. latifolium* Blue, *G. macrostachyum* Hook.f., *G. montanum* Markgraf., *G. scansens* were listed in “A checklist of the trees, shrubs, herbs and climbers of Myanmar” by Kress *et al.* (2003).

Moreover, Kress *et al.* (2003) reported that the species *Gnetum gnemon* L. was found Thaninthyi Region only. In this research, the species, *Gnetum gnemon* L. was found as a wild plant in Ye-phyu Township, Launglon Township and Dawei Township of Thaninthyi Region.

Barua (2015) described that the young leaves, tender tips and inflorescences of *Gnetum gnemon* L. were harvested from the wild for consumption, mostly by Karbi tribe in Indonesia. In this study, local people from selected area said that the young shoot of this plant could be used as vegetable crop.

In morphological study, plant *Gnetum gnemon* L. are dioecious evergreen trees, the leaves are simple, opposite, reticulate venation, exstipulate, 5-9 rings of male flowers develop basipetally above each collar, 6-9 rings of female flowers and the seeds are drupe. These characters are agreement with Larsen (1975) and Zhu *et al.* (1995). The spikes are axillary or terminal. These characters are agreed with Hooker (1885) and Kirtikar and Basu (1973). Male spikes contain male flowers and a single ring sterile female flowers, male flowers are claw-shaped, stamen one. These characters are in agreements with Larsen (1975).

The lamina of *Gnetum* has a well-marked cuticle on epidermal cells and short palisade cells and a well-developed spongy tissue. Stellately branched sclereids occur near the lower epidermis. These characters are agreement with those given by Biswas and Johri (1997).

In transverse section, the stems are circular outline, cuticle is thick and the outer walls of the epidermal cells are also very thick. The cortex layer which is differentiated into an outer chlorenchymatous, a middle parenchymatous and an inner sclerenchymatous region. Outer chlorenchymatous cells contain chloroplast and intercellular spaces. Middle parenchymatous cells in cortex region that consists of few layers of thin-walled cells. The endodermis and pericycle layers are not distinct.

In this study, the vascular bundles of the seed coat are collateral and closed type, asterosclereids are found stems and roots as well as in seed coat. Rodin (1966) reported that the asterosclereids, which is very common in petiole, midrib and larger veins of *Gnetum gnemon* L.

In present study, pitted non-lignified tracheary elements occur in lamina, cortex region of petiole, midrib, stems and roots of *Gnetum gnemon* L.. Carlquist and Goose (1995) suggested that circular bordered pits of tracheary element of *Gnetum* represents a clearly gymnosperm feature rather than on transitional to angiosperms.

In this research, the histology of seed coat comprises the parenchymatous sarcotesta region, sclerenchymatous sclerotesta and starch containing endotesta region. These characters are in agreement with those stated by Biswas and Johri (1997) and Vasishta *et al.* (1939). In

conclusion, this study has highlighted detailed descriptive information of *Gnetum gnemon* L. The presence of asterosclereids and pitted non lignified tracheary elements distribution in the whole plant could be useful in providing taxonomic information and could also serve as a database for future references of the species. In conclusion, this study has highlighted detailed descriptive information of *Gnetum gnemon* L.

### Acknowledgements

The authors acknowledge to Dr Aye Pe, Professor and Head, Department of Botany, University of Yangon, for providing the departmental facilities.

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