

## STUDY ON MORPHOLOGICAL AND HISTOLOGICAL CHARACTERS OF *BIXA ORELLANA* L.

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### Abstract

The seeds of *Bixa* produce one of the dyes most frequently used worldwide, not only in food products but also in textile, paint and cosmetic industries. The present study was conducted to investigate the morphological and histological study of *Bixa orellana* L. According to the results, it was found that the plants were small tree (6.0-8.0 ft), leaves simple, pedicellate, inflorescence terminal branched panicles with 5-15 flowered, flowers actinomorphic, hypogynous, style thickened upward, stigma bi-lobed, parietal placentation, red-brown seed pods covered in soft pines, bi-valved, seeds are obovoid and angular, red-dotted. The distinguished histological characters of *Bixa* revealed that anomocytic stomata present on both surface, druses-shaped crystal and secretory cavities occurred in lamina, petiole, midrib and cortex of stem as well as in roots. Bixin pigment producing cells are commonly found in surface view of petiole. Testa of seeds is composed of thin walled epidermal cells which are filled with pigment bixin whereas tegmen region is made up of colorless sclerenchymatous layers. Starch grain are present in endospermic cells. The histological characters of *Bixa orellana* L. could provide the diagnosis of genus *Bixa*.

**Keywords** Morphological., Histological., *Bixa orellana* L.

### Introduction

The Bixaceae family is one of the smallest plant families, consisting of one genus, *Bixa*. There are five species grouped under a single genus, and the most common species is *Bixa orellana* L. (Srineraja, 2015).

*Bixa orellana* L. is known as Annatto or Po-thidin in Myanmar, Kam-ngor, Kham-saet, Kham-ngo in Thai, Hong-mu in China, Hiryu-sida, Okenoki in Japan, Kunyit-jawa, Kesumba, Jarak-belanda in Indonesia and Sotis, Echuate in Philippines.

*Bixa orellana* L. is widely known for its dye, food coloring agents, dyeing the cloth and painting the skin. (Kala *et al*; 2015). Ayurveda practitioners in India use Annatto as an astringent, mild purgative and are considered as a good remedy for treating dysentery and kidney diseases. Traditional healers in Colombia have also used annatto as an antivenom for snake bites (Dunham and Allard, 1960).

However, the scientific investigations on this plant is still lacking in Myanmar. Therefore, the aim of the present study is to identify and examined the histological characters of *Bixa orellana* L. from Loikaw Township, Kayah Division.

### Materials and Methods

#### Morphological study of *Bixa orellana* L.

The plants used in this research were collected from Loikaw Township (North latitudes 19°42' 4" and East Longitudes 97° 12' 2"), during the flowering and fruiting period from June to November, 2020-2021. The collected specimens were identified by using standard literatures such as Backer, 1963; Hooker, 1885; Kirtikar and Basu, 1973; Flora of China, 1995; Flora of Hong Kong, 2007; Kress *et al.*, 2003.

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Microscopical study of leaves, stems, roots, fruits and seeds were also examined by using free hand sections according to the method of Esau (1963); Kokate (1994) and Trease and Evans (2002). Then the cut sections were examined by light microscope. Free hand sections were taken, stained and mounted in Safranin Reagent and chloralhydrate (50%) solution. The photographs were taken by a Camera Attached Microscope. The following reagents were used for microscopy and histological examination.

1. Chloral hydrate solutions as clearing agent
2. Phloroglucinol was followed by concentrated hydrochloric acid for lignin
3. Acetic acid or 10% sulphuric acid for calcium oxalate crystals,
4. Iodine solution B.P for starch.

### Stomatal Index.

The average number of stomata per square millimeter of epidermis is termed the stomatal number. The percentage proportion of the ultimate divisions the epidermis of a leaf which have been converted into stomata is termed the Stomatal Index.

S. I = Stomatal Index

S = number of stomata per unit area

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

### Diagnostic characters of powdered seeds of *Bixa orellana* L.

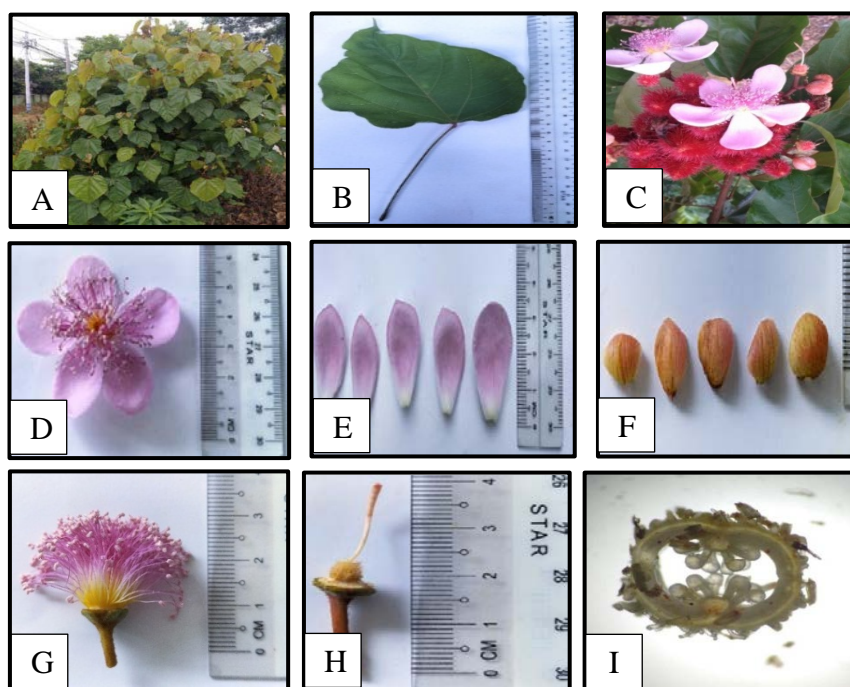
The diagnostic characters of the powder and sensory characters were examined by using the powdered seed.

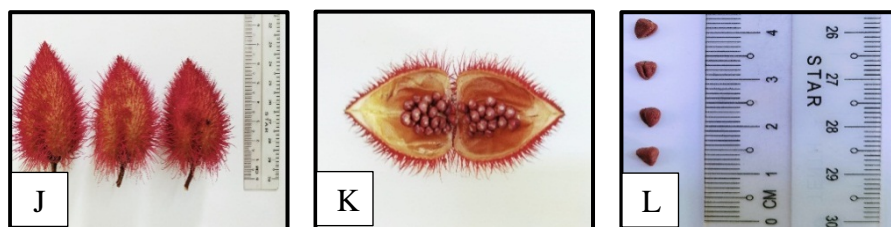
### Results

<b>Scientific name</b>	: <i>Bixa orellana</i> L.
<b>Family</b>	: Bixaceae
<b>Myanmar names</b>	: Po – thidin, Thidin
<b>English names</b>	: Annatto, Lipstick – tree
<b>Flowering period</b>	: June to November

### Taxonomic Description

Perennial, small tree, about 6 - 8 ft high; bark dark brown. Leaves simple, alternate, stipulate, petiole 4.5 - 9.5 cm long, laminae ovate, the tips acute, the margins entire, the base cordate, reticulate and netted venation, glabrous, 5.6 - 18.5 cm long, 3.7 - 13.7 cm wide. Corymbose panicles 5 - 15 flowered, scaly, peduncles 3.2 - 5.5 cm long. flowers 3.5 - 5.4 cm in diameter, purplish - pink, pedicel 2.8 - 3.2 cm long, 0.8 - 1 mm wide, bisexual, actinomorphic, 5 merous, hypogynous. Sepals 5, concave, reddish- brown, 0.3 - 0.5 cm long, more or less fleshy. Petals 5, unequal, large and conspicuous, obovate, imbricate, purplish-pink, 2.5 - 3.3cm long, 1.3 - 1.6 cm wide. Stamens numerous, slightly united at the base, filaments filiform, with yellow base and purplish-pink apex, 0.8 - 2.0 cm long, anther dithecous, basifixed. Stamens numerous, slightly united at the base, filaments filiform, with yellow base and purplish-pink apex, 0.8 - 2.0 cm long, anther dithecous, basifixed. Fruits capsules, loculicidally 2-valved, ovoid, 4.7 - 5.5 cm long, 3.8 - 4.5 cm wide, reddish-brown, covered with soft pines. Seeds many, smooth, angular, with flattened apex, orange- red, 0.3 - 0.5 cm long, 0.2 - 0.3 cm wide





**Figure 2** Morphological Characters of *Bixa orellana* L.

- |                  |                    |                   |                    |
|------------------|--------------------|-------------------|--------------------|
| (A) Habit        | (B) Leaf           | (C) Inflorescence | (D) Flower as seen |
| (E) Calyx        | (F) Corolla        | (G) Androecium    | (H) Gynoecium      |
| (I) T.S of ovary | (J) Fruits as seen | (K) Opened fruit  | (L) Seeds as seen  |

### Histological Characters of *Bixa orellana* L.

#### Lamina

In surface view of lamina, the epidermal cells of both surfaces are parenchymatous, thin-walled, irregular shape. The anticlinal walls of lower epidermal cells are wavier than the upper epidermal cells, stomata are present on both surfaces, the guard cells are reniform in shape with abundant chloroplast. Calcium oxalate crystals occur along the veins of lower surface.

In transverse section, the single layer of epidermal cells is covered by fairly thick cuticle. The mesophyll layer comprises a single layer of palisade mesophyll cells and 2-3 layers of spongy mesophyll cells. The druses –shaped calcium oxalate crystals occur below the upper epidermal layer. The vascular cylinder is collateral type, xylem composed of vessel elements, fibers, tracheid, xylem parenchyma and phloem composed of sieve tube, companion cell and phloem parenchyma.

#### Midrib

In surface view of midrib, the epidermal cells are thin walled, parenchymatous and polygonal shaped. The bixin pigment producing cells and calcium oxalate crystals are also occurred.

In transverse section, the upper region is convex and inner is concave. The thick wall epidermal cells are compactly arranged. The hypodermis layer lies next to the epidermis and consists of 3-5 layers of collenchymatous cells, 5-7 layers of parenchymatous cells whereas 2-4 layers of collenchymatous cells and 7-10 layers of parenchymatous cells are occurred in abaxial region of transverse section of midrib. The vascular bundle is surrounded by sclerenchymatous sheath. The xylem towards centre and the phloem towards periphery. Moreover, the lysigenous cavities and calcium oxalate crystals occurred in adaxial and abaxial region of midrib.

#### Petiole

In surface view of petiole, the epidermal cells are thin-walled, rectangular in shape. The bixin pigment producing cells and calcium oxalate crystals are occurred in surface view of petiole.

In transverse section of petiole, single layer of epidermal cells are barrel in shaped. The cortex region is made up of 2-3 layered of collenchymatous cells and 5-8 layered of parenchymatous cells and become larger towards central region. The vascular bundle is concentric and amphicribal type. The lysigenous cavities and crystals are found in the cortex region as well as in the region of petiole.

## Stem

In surface view, the epidermal cells are thick wall and rectangular shape. In transverse section, the outline of stem is rounded. The phellem is 1-2 layers, phloem consists of 2-5 layered of cells. The phelloderm is composed of 2-3 layers of parenchymatous cells. The central vascular bundles are found as collateral type. The pith is present and made up of parenchymatous cells.

## Root

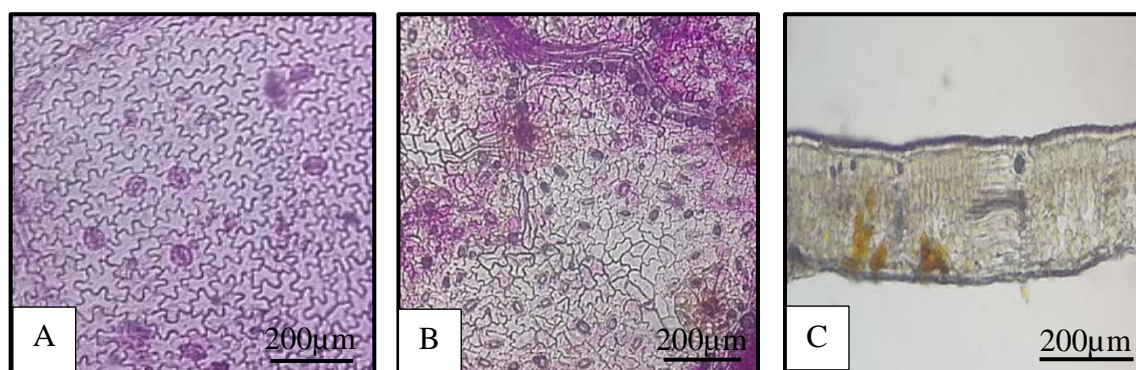
In surface view, the epiblema cells are rectangular in shape. In transverse section of root, the epiblema cells are thin-walled and the cortex region consists of thin walled, isodiametric parenchymatous cells, the sclereids are occurred as patches in cortex region. The innermost layer of cortex is endodermis. The vascular bundle shows radial arrangement, xylem elements show exarch condition and the phloem structure is similar to those of stem.

## Seed

In surface view, the epidermal cells are polygonal and their walls are straight. In transverse section, the seed coat is differentiated into two regions. The testa region is composed of thin wall epidermal cells, 1-2 layers thick which are filled with pigment bixin. Next layer is single layer of closely packed, colorless palisade cells and the tegmen is composed of two regions. The outer region is the pigment zone and contain 1-2 layers of sclerenchymatous cell. Next to this layer is 2-3 layers of colorless sclerenchymatous layer and made up of osteosclereids. The endosperm is composed of parenchymatous cells which were densely filled with starch grain.

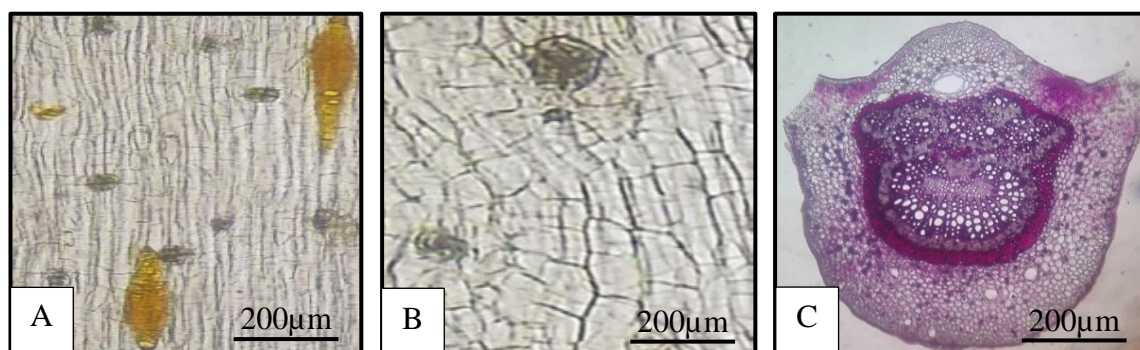
### Diagnostic characters of powdered *Bixa orellana* L.

The powdered seeds were orange red in color, the odor was pungent, the taste was slightly nutty and the texture was fine. It consists of fragment of fiber and reticulate vessel, fragment of osteosclereids and group of starch grains.

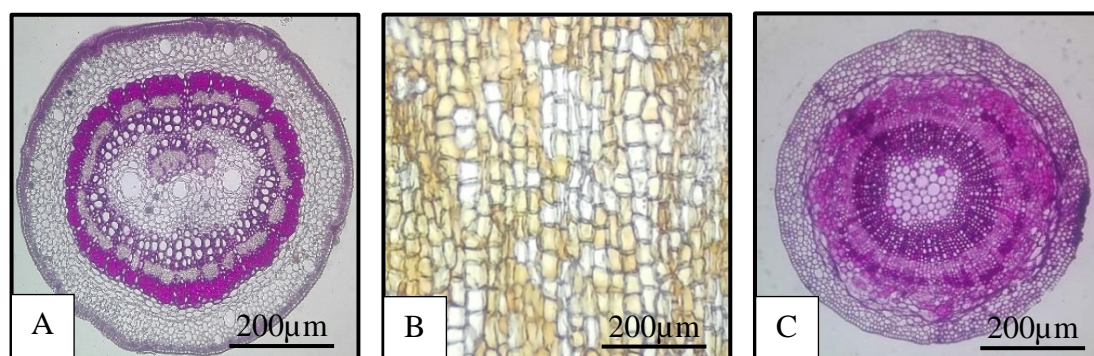


**Figure 3** Internal Structures of lamina of *Bixa orellana* L., (A) Upper surface of lamina, (B) Lower surface of lamina, (C) Transverse section of lamina

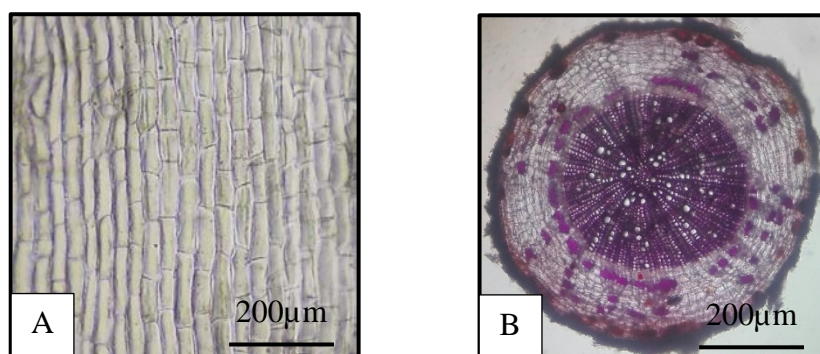




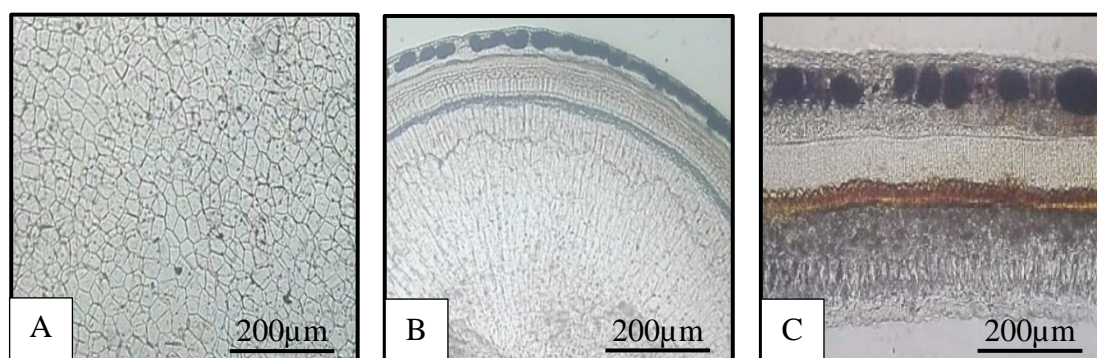
**Figure 4** Internal Structures of midrib of *Bixa orellana* L. (A) Surface view of midrib, (B) Surface view of midrib showing a calcium oxalate crystal, (C) Transverse section of midrib



**Figure 5** (A) Transverse section of petiole, (B) Surface view of stem (C) Transverse section of stem



**Figure 6** (A) Surface view of root, (B) Transverse section of root



**Figure 7** Internal Structures of Seed of *Bixa orellana* L., (A) Surface view of seed coat, (B) A portion of T.S of seed coat, (C) An enlarge cellular portion of testa



**Figure 8** Diagnostic Characters of Powdered Seeds of *Bixa orellana* L., (A) Starch grains treated with iodine, (B) Fragment of fibre and reticulate vessel, (C) Fragment of osteosclereids

### Discussion and Conclusion

In this research, the morphological and histological characters of *Bixa orellana* L. (Family –Bixaceae) have been undertaken.

In Myanmar, there is only one genus and one species in family Bixaceae (San Khin, 1970 and Kress *et al.*, 2003).

*Bixa orellana* L. is a perennial small tree, leaves simple, petiole erect, flowers 10-20 in corymbose panicles, sepals and petals 5, free, imbricate, stamens many, anthers 2-celled, ovary superior, unilocular, parietal placentae, stigma bilobed, capsule 2-valves and seeds many. These characters are in agreement with Hooker (1894), Kirtikar (1935), Backer (1963) and Dassanayake (1981).

In the present study, anomocytic stomata present on both surface, druses-shaped crystal and secretory cavities occurred in lamina, petiole, midrib and cortex of stem as well as in roots.

Metcalf and Chalk (1950) described that the lamina of genus *Bixa* had anomocytic stomata that occurred on both surfaces, secretory cavities and cluster crystals of calcium oxalate present in the mesophyll and in the peripheral tissue of the petiole.

In transverse section of seeds, the reddish-brown pigment is found in testa region of seed coat of Annatto. The color of seed coat in *Bixa orellana* L. varies from orange to reddish brown, due to the carotenoid content, which is mostly found in the aril.

*Bixa* seeds has a- bixin, b-bixin, a- norbixin and b-norbixin carotenoids. Moreover, the minor apocarotenoid pigment are also present. (Reith 1971, Mercadante *et al.*, 1999).

In conclusion, *Bixa orellana* L. in Bixaceae family could be identified based on microscopically characteristics of leaves, stems, roots and seeds.

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