

# A Comparative Anatomical and Epidermal Analysis of *Physalis angulata* L. and *Physalis micrantha* L. (Solanaceae)

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## Abstract

The epidermal and anatomical characteristics of *Physalis angulata* L. and *Physalis micrantha* L. (Solanaceae) are analyzed in this study to determine the taxonomic features of these species. The specimens were peeled or sectioned, stained with safranin O and alcian blue, mounted on slide, and micro-photographed with Optika B-1000 FL LED. The plants are amphistomatic and dorsiventral with anisotricytic, isotricytic, tetracytic, anisocytic, anomocytic and contiguous stomata. *P. micrantha* has calcium oxalate crystals of different forms (prism, rod, cylindrical, and star-shaped or druses), and osteosclereide, while *P. angulata* has only druses and macroscleried occurring in parts of the plants. The similarities in the anatomical features among these species suggest an interspecific relationship between the species while the differences confirm these species as distinct.

**Keywords:** Amphistomatic, Druses, *Physalis*, Prismatic crystals, Solanaceae.

## 1. Introduction

*Physalis* L. belongs to the family Solanaceae. *Physalis* is believed to have originated in Mexico (Kelly *et al.*, 2012). Generally, there are at least seventy-five known species of the genus *Physalis* (Whitson and Manos 2005). In West Tropical Africa, only four species are known; namely, *P. angulata* L., *P. peruviana* L., *P. micrantha* L. and *P. pubescens* L. (Burkill, 2000). Among these species, *P. pubescens* is only found in Ghana, and is native to America (Burkill, 2000), while the other three species *P. angulata*, *P. micrantha* and *P. peruviana* are widely distributed in Nigeria (Olorode *et al.*, 2013).

The importance of epidermal, anatomical, morphological and cytological characteristics in taxonomy of vascular plants have been investigated and recognized by several authors (Hutchinson and Dalziel, 1954; Metcalfe and Chalk, 1979; Wahua and Sam, 2013; Olorode *et al.*, 2013; Ekeke and Mensah, 2015; Ekeke and Agogbua, 2017). The anatomical, epidermal and morphological characteristics of the members of Solanaceae have also been reported (Hutchinson and Dalziel, 1954; Metcalfe and Chalk, 1979; Olorode *et al.*, 2013; Wahua and Sam, 2013; Chockpisit and Aree, 2013). The morphological, floral and cytological characteristics of these species have also been described (Olorode *et al.*, 2013).

Information on the anatomical features of the studied species is scanty. This research is carried out to outline the epidermal and anatomical characteristics of these species to complement the existing data on the species.

## 2. Materials and Methods

### 2.1. Plant Source and Identification

The plant species investigated in this study are *Physalis angulata* L. and *P. micrantha* L. Samples of these plants were collected from various locations in Choba, Nigeria, between April and December of 2016 and November 2017 when the plants were in full bloom and in their optimal conditions. The collected samples were identified at the herbarium of the University of Port Harcourt.

### 2.2. Epidermal Studies

For the epidermal analysis, foliar sections were collected from fresh plants growing in fields. One square centimeter leaf cuttings were obtained from identical regions of each fresh leaf, generally mid-way between the leaf base and apex of the lamina. The adaxial and abaxial epidermal peels were obtained using sharply pointed forceps. Peels were stained with 1 % safranin O and alcian blue, rinsed with distilled water to remove excessive stain and were then mounted in a drop of pure glycerol on clean glass slides. A cover glass was placed over the drop and sealed with nail varnish to prevent dehydration (Okoli and Ndukwu, 1992). The epidermal features observed included: the organization of the epidermis, the arrangement of the epidermal cells, the nature of trichomes, the shape of epidermal cells, and the nature of the anticlinal cell wall of the leaf epidermis, stomatal types, and stomatal density and index. The stomatal index (SI) was estimated based on (Metcalf and Chalk, 1979),

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while the terminology for the stomatal type is based on (Malvey, 2004).

### 2.3. Anatomical Studies

Fresh samples (petiole, stem and midrib) of the *Physalis* species were fixed in formalin, acetic acid, and alcohol (FAA) for twelve hours, dehydrated in alcohol series (30 %, 50 % 70 %, 95 % and absolute alcohol) for three hours each, cleared in chloroform-alcohol series ((3:1; 1:1; 1:3) v/v for ten minutes in each, wax-embedded and sectioned. Thin sections were selected, de-waxed, stained with Alcian blue, and counterstained with safranin (Okoli and Ndukwu, 1992). Good preparations were mounted on slides; they were viewed and photographed with Optika B-1000 FL LED

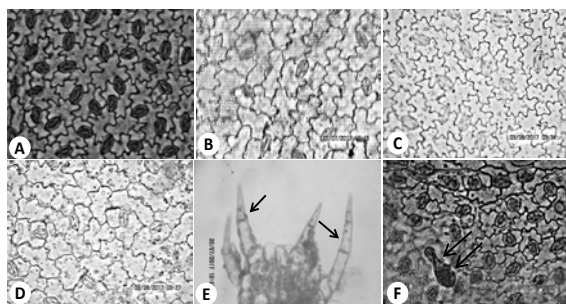
## 3. Results

### 3.1. Leaf Epidermal Characteristics

The two species are amphistomatic and have dorsiventral leaves. The leaf epidermal characteristics slightly varied among them with the non-glandular, multicellular base, uniseriate trichomes with one-four celled stalk (Fig. 1E) and glandular trichomes; the base is multicellular, the stalk has one-two cells and the globular multicellular head, three-four cells. (Figure 1F) and Table 1.

#### 3.1.1. *Physalis micrantha*

The upper and lower epidermal cells are irregular in shape with undulating anticlinal cell walls (Figure 1A-B) and stomatal indices of 88.23 and 38.09 on the lower and upper epidermis respectively. The upper epidermis had isotricytic, tetracytic, anisocytic but rarely anomocytic stomata (Figure 1A) while the lower epidermis had isotricytic, tetracytic, tricytic and polar contiguous stomata with glandular trichomes (Figures 1B and 1F).



**Figure 1. Epidermal characteristics of *Physalis* species:** (A- upper and B-lower) *P. micrantha* and (C- upper and D- lower) *P. angulata*. E- arrows show non-glandular multicellular uniseriate trichomes and F- arrows show glandular trichome on *P. micrantha* epidermis.

#### 3.1.2. *Physalis angulata*

The upper and lower epidermal cells of this species are irregular in shape with undulating anticlinal walls and glandular trichomes (one-two- celled stalk, multicellular base, three-four-celled head). The upper epidermis had anisotricytic, isotricytic, tetracytic and anisocytic stomata (Figure 1C) with a stomatal index of 40.00. The lower epidermis had anisotricytic, isotricytic, tetracytic, anisocytic and anomocytic (Figure 1D) and a stomatal index of 76.47.

**Table 1.** Leaf epidermal characteristics of the studied *Physalis* species.

Plant part	<i>P. angulata</i>	<i>P. micrantha</i>
Lower epidermis	Irregular in shape, anticlinal wall undulating, stomata (anisotricytic, isotricytic, tetracytic, anisocytic and anomocytic) with glandular trichomes and a stomatal index of 76.47.	Irregular in shape, anticlinal wall undulating, stomata (isotricytic, tetracytic, tricytic and polar contiguous) with glandular trichomes and a stomatal index of 88.23
Upper epidermis	Irregular in shape, anticlinal wall undulating, stomata (anisotricytic, isotricytic, tetracytic, anisocytic) with glandular trichomes and a stomatal index of 40.0.	Polygonal in shape, anticlinal wall undulating, stomata (isotricytic, tetracytic, anisocytic but rarely anomocytic) with macrosclereid and a stomatal index of 38.09.

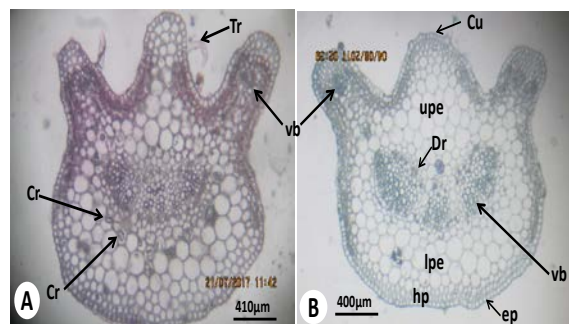
### 3.2. Anatomy of Petiole, Midrib, Lamina and Stem

#### 3.2.1. *Physalis micrantha*

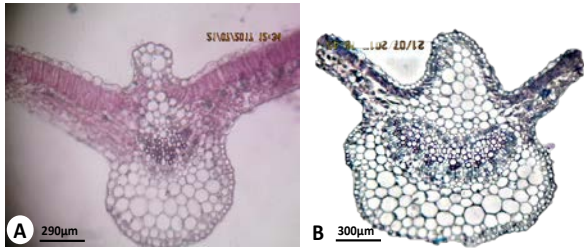
Petioles are hairy. The epidermis has two-layers. The upper surface has four-six layers of a parenchymatous cell with three separate arced vascular bundles (Figure 2A). The midrib is hairy. The upper cuticle protruded or projected with a relatively flat surface. The vascular bundle is small forming a semi-arc with parenchymatous cells of three-two layers on the lower surface and six-nine layers on the upper surface (Figure 3A). Lamina has one-layered palisade mesophyll (Figure 4A). The stem is oval with six protruded ends. Pith is small and hollow; the epidermis has one-layer and a hollow pith of about 245  $\mu\text{m}$  wide (Figure 5A).

#### 3.2.2. *Physalis angulata*

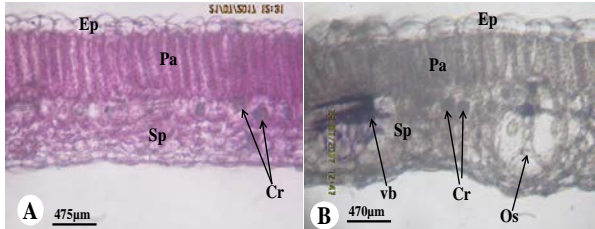
Petioles are hairy on both upper and lower surfaces. The epidermis has layers and one layer of hypodermis and vascular arced (Figure 2B). The midrib is hairy, with arced vascular bundles, parenchymatous cells of three-four layers on the lower surface and three-eight layers on the upper surface. The lower epidermis has one layer and one layer of hypodermis (Figure 3B). Lamina has one layer of palisade mesophyll and the vascular bundles are embedded in the spongy mesophyll or between the palisade and spongy mesophyll (Figure 4B). The stem is fairly rectangular in shape with five protruded ends; the epidermis has one layer and a hollow pith of about 375  $\mu\text{m}$  wide (Figure 5B).



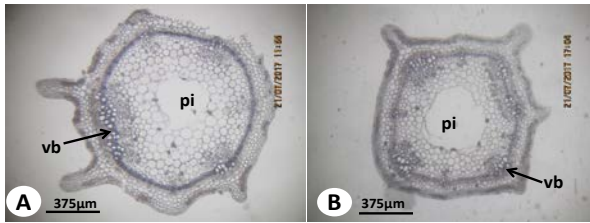
**Figure 2. Petiole anatomy of *Physalis* species:** (A) - *P. micrantha* and (B) - *P. angulata* (Cr-crystal, vb-vascular bundle, Tr-trichome, Cu-cuticle, upe-upper parenchymatous cells, lpe-lower parenchymatous cells, hp-hypodermis, ep-epidermis, Dr-druses).



**Figure 3.** Midrib anatomy of *Physalis* species: (A) - *P. micrantha* and (B) - *P. angulata*.



**Figure 4.** Anatomy of leaf lamina of *Physalis* species: (A) - *P. micrantha* and (B) - *P. angulata* (Ep-epidermis, Pa-palisade mesophyll, Sp-spongy mesophyll, Cr-crystal, Os-osteosclereide).



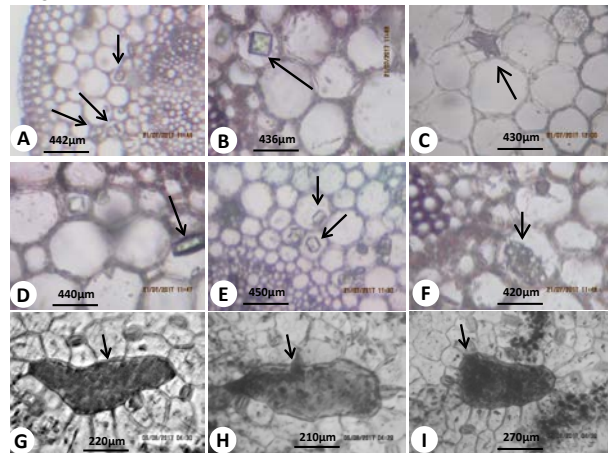
**Figure 5.** Stem anatomy of *Physalis* species: (A) - *P. micrantha* and (B) - *P. angulata* (pi-pith, vb-vascular bundle).

**Table 2:** Anatomy of petiole, midrib, lamina and stem of the *Physalis* species.

Plant part	<i>P. angulata</i>	<i>P. micrantha</i>
Petiole	Hairy on both surfaces, epidermis two-layered or having one layer of hypodermis	Winged with vascular bundles, the upper epidermis has two layers of collenchyma, 4-6 layers of parenchyma, one layer of collenchyma.
Lamina	Mesophyll one-layered, vascular bundles embedded in the spongy mesophyll or between the palisade and spongy mesophyll.	One layer of palisade mesophyll and osteosclereide embedded in the spongy mesophyll but extending to the lower epidermis.
Midrib	Hairy with arced vascular bundles, parenchymatous cells 3-4 layers on the lower surface and 3-8 layers on the upper surface, the lower epidermis has one layer and one layer of hypodermis	Upper cuticle protruded/projected with a relatively flat surface having 6-9 layers of parenchymatous cells. Lower parenchyma 3-5 cells with non-glandular serrated hairs.
Stem	Rectangular in shape with 5-protruded ends, the epidermis is one-layered.	Oval in shape with six-protruded ends, pith hollow, with a continuous layer of sclerenchymatous cells.

### 3.3. Distribution of Calcium Oxalate, Secretory Cells, and Sclereides

Among the *Physalis* species studied here, the researchers observed calcium oxalate crystals and sclereids of different forms in the stem, petiole, midrib, and lamina (Figures 4 B and 6, and Table 3). The different forms of calcium oxalate include: pyramidal (Figure 6 B), rod (Figure 6 D), druses or star-shaped (Figures 6 C and 6 F) and cylindrical (Figure 6 E) while the sclereids are macrosclereides (Figures 6 G-6 I) and osteosclereide (Figure 4 B).



**Figure 6.** (A – E) *Physalis micrantha* Petiole, (F) *P. angulata* (G-I) - Macrosclereides on Upper epidermis of *P. micrantha* (A-prismatic crystals; B-pyramidal; C and F- druses or star-shaped; D - rod and E – cylindrical).

#### 3.3.1. *Physalis micrantha*

Druses were found in the spongy and palisade mesophylls in the lamina (Figure 4A), in the parenchymatous cell towards the lower epidermis, in and around the vascular bundles in the midrib, and predominantly in the cortex in the stem, while druses and prismatic crystals (cylindrical, pyramidal or rod shape) were found in the petiole (Figures 2A and 6A-E) and macrosclereides in the upper epidermis (Figures 6G – 6I).

#### 3.3.2. *Physalis angulata*

Calcium oxalate crystals (druses) and secretory cells were observed mainly in and around the vascular bundles in the petiole (Figure 2B) and the midrib. Also, crystal sand was in the upper and lower parenchymatous cells in the midrib. In the lamina druses were found in the spongy mesophyll, and palisade mesophylls, osteosclereide embedded in the spongy mesophyll, but extending to the lower epidermis (Figure 4B) and druses predominantly in the cortex and the parenchymatous cells in the stem.

**Table 3.** Distribution of calcium oxalate, secretory cells and sclereids in the studied plants.

Plant part	<i>P. angulata</i>	<i>P. micrantha</i>
Petiole	Calcium oxalate crystals (druses) and secretory cells found in and around the vascular bundles.	Calcium oxalate crystals (druses, and prismatic crystals-cylindrical or rod shape).
Lamina	Druses found in the spongy and palisade mesophylls, osteosclereide embedded in the spongy mesophyll but extending to the lower epidermis.	Druses found in the spongy and palisade mesophylls.
Midrib	Mainly druses and crystal sand in the upper and lower parenchymatous cells, vascular bundles surrounded by secretory cells.	Druses found in the parenchymatous cell towards the lower epidermis and in and around the vascular bundles.
Stem	Druses predominantly found in the cortex and the parenchymatous cells.	Druses predominantly found in the cortex, spherical in shape.

#### 4. Discussion

The importance of epidermal, anatomical, morphological and cytological characteristics in taxonomy of vascular plants have been investigated and recognized by several authors (Hutchinson and Dalziel, 1954; Metcalfe and Chalk, 1979; Olorode *et al.*, 2013; Wahua and Sam, 2013; Ekeke and Mensah, 2015; Ekeke and Agogbua, 2017). The anatomical, epidermal and morphological characteristics of the members of Solanaceae including *Physalis* have been reported (Hutchinson and Dalziel, 1954; Metcalfe and Chalk, 1979; Olorode *et al.*, 2013; Wahua and Sam, 2013; Chockpisit and Aree, 2013). Chockpisit and Aree (2013) recognized that the epidermal cells of *Physalis* species are irregular in shape, with undulating anticlinal walls. Also, they reported that the spongy mesophyll has three-four layers and the palisade mesophyll has one layer. This is consistent with the observation in the current study.

The current findings state that the leaves of both *P. angulata* and *P. micrantha* are amphistomatic and dorsiventral, which is similar to the report of Sethi and Kannabiran (1975) with isotricytic, tetracytic, anisocytic, anomocytic, tricytic, anisotricytic and polar contiguous stomata. Sethi and Kannabiran (1975) further noted that the stomata type in *Physalis* was anomocytic, while Sandhya, *et al.* (2010) reported that the stomatal apparatus observed in *P. angulata* was of the anisocytic type. Zhang and Lu (1999) reported that anomocytic stomata were restricted only to the abaxial surface of the leaf in *P. angulata*. This observation is different from the observation regarding these species in this study. Chockpisit and Aree (2013) observed stomatal indices of 3.57 and 4.00 on the adaxial and abaxial surfaces of *P. angulata*, while Wahua and Sam (2013) observed stomatal indices of 20.0 and 13.64 on the adaxial and abaxial surfaces of the same species. The observation from this study is different because other stomata types along with the anomocytic and anisocytic types were observed. The stomatal index found in this study also differed from the

previous reports. Wahua and Sam (2013) further reported uniseriate non-glandular and glandular trichome in both *P. angulata* and *P. micrantha* which corresponds with the current observation.

#### 5. Conclusion

The difference in morphology and occurrence of calcium oxalate crystals and sclereid among these species is worthy of note. Druses and prismatic crystals (cylindrical, pyramidal or rod-shaped) and macrosclereides were found in *P. micrantha*, but only druses and osteosclereide were found in *P. angulata*. The variation confirms that these species are distinct as reported by Hutchinson and Dalziel (1954) and Olorode *et al.* (2013). The similarities in the anatomical and epidermal characteristics among the species suggest an interspecific relationship between the species, while the differences confirm the species to be distinct.

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