

# Floral and Leaf Anatomy of *Hibiscus* Species

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**Abstract** Comparative anatomical studies of the leaves and flowers of *H. arnottianus*, *H. surattensis*, *H. acetosella* and *H. rosa-sinensis* are described. The anisocytic stomata was the commonest followed by brachyparacytic, anomocytic, staurocytic stomata and laterocytic stomatas respectively. *H. acetosella* are distinguished on other species by having laterocytic stomata on both surfaces of leaves and parallel contiguous stomata are found on abaxial surface while in *H. rosa-sinensis* laterocytic is found only on adaxial surface. There are five different types of abnormal stomata, unopened stomatal pore, two stomata sharing one subsidiary cell, parallel contiguous stomata and aborted guard cell found in all the surfaces of the leaves and flowers. In addition parallel contiguous stomata are found on adaxial surface of *H. rosa-sinensis* and abaxial surface of *H. arnottianus* flower. *H. rosa-sinensis* had five-armed trichome on the abaxial surface that helps in distinguishing it from other species studied. Crystal druses are only present on both adaxial surface of *H. arnottianus* and *H. rosa-sinensis* leaf and on the abaxial surface of *H. acetosella* flower. The shape of epidermal cells, anticlinal cell walls, guard cell areas, stomatal index and trichomes varied. The results obtained could be used as diagnostic tool for plant identification and preparation of monograph on the species.

**Keywords:** *Hibiscus*, *Malvaceae*, guard cell area, stomatal index, trichome, anticlinal cell walls

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## 1. Introduction

The family Malvaceae is one of the most important families consisting of 82 genera and 1,500 species with *Hibiscus* over 200 species, *Sida* 200 species, *Abutilon* 190 species and *Malva* 40 species. The family is world wide in distribution but is mostly represented in the tropical and subtropical region. Members may be herbs, shrub or the trees with mucilage. The plants *Hibiscus rosa-sinensis*, *Hibiscus surattensis*, *Hibiscus arnottianus* and *Hibiscus acetosella* belong to the sub-kingdom *Tracheobionta* (vascular plants). Division *Magnoliopsida* and family Malvaceae [1].

*Hibiscus, rosa-sinensis*, young leaves are sometimes used as a spinach substitute. Flowers of *Hibiscus rosa-sinensis* can also be made into a kind of pickle or used as a purple dye for colouring foods such as preserved fruit and cooked vegetables. Root is edible but very fibrous. *H. rosa-sinensis* flower preparations are used for hair care. The flowers themselves are edible and are used in salads in the Pacific Islands. The flowers are used to shine shoes in parts of India. The red flowers of *Hibiscus acetosella* and possibly also the leaves are occasionally used to make a tea, somewhat similar to the use of the red calyces of *Hibiscus sabdariffa*. The root of *H. acetosella* is edible but insipid and fibrous. Pink – or red flowers are often grown as ornamental plants in gardens. Some people in Cameroon and Democratic Republic of Congo combine the use of *Hibiscus acetosella* as a vegetable with its use

as a hedge to separate plots. A red leaved cultivar of *H. acetosella* is also grown for its attractive foliage [2].

*Hibiscus sabdariffa* is said to lower fevers and high blood pressure, relieve coughs, increase urination and kill bacteria. China rose (*H. rosa-sinensis*) is used primarily for respiratory problems but also for skin disorders and to treat fevers. *Hibiscus syriacus* (rose of sharon) is used externally as an emollient to soften and soothe the skin as well as used internally for digestive disorder [3].

The essential oil in the seeds of *H. rosa-sinensis* has a strong antispasmodic effect and has been successfully used to ease the pain for intestine pile or kidney colic. The flower extract is used internally in the treatment of excessive and painful menstruation. Venereal diseases and to promote hair growth [4] *Hibiscus rosa-sinensis* extract exerted potential protective effect against tumor. Promotion stage of cancer development [5] and the anthocyanins extract of this plant grown in Egypt proved pronounced cytotoxic activities against hepatoma and breast cancer cell lines [6].

The leaves of *H. rosa-sinensis* are emollient, aperients, anodyne and laxative, leaves and stem bark are used for abortion. The staminal column is diuretic used for kidney trouble. The flowers are astringent, demulcent, emollient, refrigerant, constipating, hypoglycaemic, aphrodisiac, emmenagogue and used for treating alopecia, burning sensation in the body, diabetes and menstrual disorders. The buds are used for treatment of vaginal and uterine discharges. Leaves and flowers are good for healing ulcers and for promoting growth and colour of hair. The leaves

and flowers of *H. rosa-sinensis* are also beaten into a paste and poulticed onto cancerous swellings and mumps.

In Angola, an infusion of the leaves of *Hibiscus acetosella* in water is used as post-fever tonic, it is also used as medicine to treat anaemia. In East Africa, children with an aching body are washed in cold water to which some mashed *Hibiscus acetosella* leaves have been added [2].

In recent times, the classification of *Hibiscus* species in Nigeria still pose some problems due to variation within taxa and high degree of hybridization. Previous pharmacological investigation of the genus *Hibiscus* indicated the presence of some species with useful biological activities as antihypertensive, anti-inflammatory, anti-pyretic, hepatoprotective, anti-diarrhoeic, anti-spermatogenic, anti-tumour, anti-diabetic, anti-convulsivant, anti-helminthic immunodulator, anti-oxidant and antimutagenic agents [7-15].

Presence of various types of glandular and eglandular trichomes is a characteristics features of genus *Hibiscus*. Scientific interest in plants trichome is based on their functional and taxonomic importance and on the economic usefulness of some trichome-generated products. Trichome may serve a variety of defensive and physiological functions. Leaf trichomes have been shown to reduce insect herbivory in a number of plant species [16,17,18].

For plants in xeric habitats, epidermal appendages reflect light and can reduce transpiration rate [19,20]. The micromorphological characteristics of foliar trichomes have played an important role in plant systematic especially of particular groups at generic and specific levels. Such type of studies in the field has fascinated plant morphologists and systematists towards the diversity of trichomes features [21].

Spring [22] stated that the presence or absence of peltate hairs and their form, size and colour could be used in distinguishing between genera and species, but also their corresponding parts, thus being important in pharmacognosy, archaeobotany, paleobotany and agronomy [23].

Adedeyi and Illoh [24] reported the comparative foliar anatomy of the genus *Hibiscus*, but they concentrated on the general anatomy and added only few sentences about foliar trichomes without any morphological descriptions and anatomical measurements. In their work, they found out that the eight major types of glandular and eglandular foliar trichomes, have been observed.

Nwachukwu and Mbagwu [25] reported on anatomical features of the roots and leaves of *Hibiscus rosa-sinensis* and *Abelmoschus esculenta* results obtained from it showed presence of long chain and numerous epidermal cells in *Hibiscus rosa-sinensis* while they are short chains small and numerous epidermal cells in *Abelmoschus esculenta*. Priya *et al.* [26] pointed out the stomatal variation in cultivars of *Hibiscus rosa-sinensis* L. Calcium oxalate crystals may form in any organ or tissue within plants, crystals occur in roots, stems, leaves, flowers, fruits and seeds [27] and within epidermal, ground and vascular tissues [28].

The study was to ascertain the taxonomic importance of flowers and leaf anatomical features in deducing similarities and differences among the taxa studied and utilizing the anatomical characters obtained from these

taxa for the systematic grouping and characterization of the taxa. The study attempted to reveal additional characteristics for *Hibiscus* species which might be useful for identification of the species studied at the generic and species level.

## 2. Materials and Methods

### 2.1. Plant Collection

The fresh leaves and flowers of *Hibiscus rosa-sinensis*, *H. arnottianus*, *H. surattensis* and *H. acetosella* were collected in September 2012 in Uyo Local Government Area of Akwa Ibom State. The plants were authenticated by Dr. (Mrs.) U. A. Essiett, a taxonomist in the Department of Botany and Ecological studies. Anatomical studies were carried out using the methods below.

### 2.2. Microscopic Examination

For the purpose of anatomical studies small sizeable portions were obtained from standard median part of the leaves of matured and well expanded leaves. Epidermal peels of both abaxial and adaxial surfaces were made by placing the leaf blade taken from standard median portion of the leaves on clean glass slab. The specimen surfaces to be studied was placed facing down; it was irrigated with water holding down the specimen from one end. The epidermis above the desired surface was scrapped off carefully with a sharp razor blade. Loose cells were washed away from the epidermal peel with the aid of soft carmel hairbrush and watered until the desired epidermis below was reached. The epidermis peels were stained in 1% aqueous solution of Safranin 0 for 4 to 8 minutes rinsed carefully in water to remove excess stain and then mounted in 10% glycerol. Guard cells area was calculated by Francos constant method (Guard cells area = length x width x 0.7854). The stomatal index was determined according to Metcalfe and Chalk (1979) using the formula:

$$\frac{S}{E + S} \times \frac{100}{1} = \text{Stomatal Index}(SI)$$

Where: S = number of stomata per unit area

E = number of epidermal cells in the same area

All measurements were made with the aid of an Ocular micrometer and finally converted by the Ocular constant with respect to the power under which they were taken. Images were computerized digitally with a motic images plus version 2.0 ml mounted on Zeiss light microscope.

## 3. Results

### 3.1. *H. arnottianus* (M. Roe) D. Bates

The leaf epidermal cell of the adaxial surface are rectangular to polygonal to irregular in shape while abaxial is polygonal to irregular. Adaxial cells (40 x 26 μm) are smaller in size than that of the abaxial (56 x 23 μm) as shown in Table 1.

Anticlinal cell walls of adaxial surface are straight to slightly undulating while abaxial surface are straight to undulating. Peltate trichome, bifurcated (two-armed) trichome, unicellular nonglandular trichome are found on

the adaxial surface while peltate trichome, four-armed sessile stellate trichome, unicellular trichome, two-armed trichome are found on abaxial surface (Plate 1: B, C, D, K, M, N and O, Table 3)

Stomata distribution in the investigated taxa is hypoamphistomatic (stomata abundant on abaxial surface and scanty on adaxial surface).

The mature stomata are mostly anisocytic, anomocytic, staurocytic and brachyparacytic (Plate 1: A, F, H, I, J, P, S and T) found on both surfaces which are distributed and their axes are oriented in different directions. Staurocytic stomata is abundant. Guard cell area on adaxial surface (5  $\mu\text{m}$ ) is higher than those of abaxial surface (4  $\mu\text{m}$ ). Abnormal stomata with unopened stomatal pore and stomata sharing one subsidiary cell are present on abaxial surface but aborted guard cells are found on both surfaces (Plate 1: G, L, Q, R). Crystal druses are common on adaxial surfaces (Plate 1 E).

The flower anticlinal cell wall of the adaxial surface are slightly undulate while abaxial surface are sinous or undulate (Table 2). The flower epidermal cell on adaxial are polygonal to irregular while abaxial is polygonal.

Stomatas are scanty on the adaxial surface and abundant on the abaxial surface (hypoamphistomatic). The mature stomata types were anisocytic, anomocytic and staurocytic on the abaxial surfaces while on the adaxial surface staurocytic stomata are mostly frequent followed by anisocytic (Plate 8: B, C, D, E, F).

The lowest guard cells were recorded in adaxial surface (5  $\mu\text{m}$ ) and the highest was found on abaxial surface (7  $\mu\text{m}$ , Table 2). Abnormal stomatas are parallel contiguous stomata on the abaxial surfaces only (Plate 8H). Glandular trichome are found on both surfaces (Plate 8: A, G, Tab. 3). Crystal druses were absent.

### 3.2 *H. surattensis* L.

Epidermal cells on the adaxial surface of the leaf are pentagonal to polygonal while abaxial surface are polygonal to irregular. Abaxial cells (65 x 30  $\mu\text{m}$ ) are smaller in size than adaxial cells (78 x 28  $\mu\text{m}$ ) as shown in Table 1. Anticlinal cell walls of adaxial surface are undulate while abaxial cells are straight to sinous. Curved trichome on vein cells, two-armed non-glandular trichome on vein cells, glandular trichome on the abaxial surface while on the adaxial surface are glandular trichome (Plate 2: C, D, K, P,U, Table 3). Stomatal distribution in the investigated taxa is hypoamphistomatic (stomatas abundant on abaxial and scanty on adaxial surface).

The mature stomatas are mostly anisocytic, brachyparacytic, laterocytic, staurocytic on both surface but in addition, anomocytic stomatas were present on the abaxial surface only (Plate 2: A, B, H, I, L, M, N, S, T). Guard cell area in adaxial surface (7  $\mu\text{m}$ ) was smaller than that of abaxial surface (8  $\mu\text{m}$ , Tab. 1). Abnormal stomata with aborted guard cells, one guard cell and unopened stomatal pore were observed on both surfaces but only 2 stomata sharing one subsidiary cell were found on abaxial surface (Plate 2: E, F, G, J, O, Q, R).

The flower epidermis of the adaxial surface are polygonal to irregular while abaxial surface are pentagonal to polygonal to irregular. The anticlinal cell walls of the adaxial epidermis are straight undulating while abaxial are undulating (Table 2). Stomatas are

completely absent on both abaxial and adaxial surfaces (Table 2). Glandular trichomes are present on only the abaxial surface (Plate 7B, Table 3). Crystal druses are absent in all the surfaces.

### 3.3 *H. acetosella* Welw. Ex Hiern.

Epidermal cells on the adaxial surface of the leaf are pentagonal to polygonal to irregular while abaxial surface are polygonal to irregular. Abaxial cells (59 x 24  $\mu\text{m}$ ) are smaller than adaxial cells (75 x 34  $\mu\text{m}$ ) as shown in Tab. 1. Anticlinal cell walls of adaxial surface are slightly undulating while abaxial surface is undulating. Few glandular trichomes are found on the abaxial surface (Plate 3F). Stomatal distribution in the investigated taxa is amphistomatic (stomatas are abundantly present on both surfaces). Mature stomatas found on abaxial surface are anisocytic, staurocytic, and laterocytic while anisocytic, brachyparacytic, few anomocytic and laterocytic stomatas are found on the adaxial surface and anisocytic are more abundant (Plate 3: A, B, J, K, M). Guard cell area in abaxial (5  $\mu\text{m}$ ) was smaller than that of adaxial (14 $\mu\text{m}$ , Table 1). Abnormal stomata with aborted guard cell, one guard cell and unopened stomatal pore were observed on both surfaces while parallel contiguous stomata and two stomata sharing one subsidiary cell were found only on the abaxial surface. (Plate 3; C, D, E, G, H, I, L, N). Crystals were completely absent.

The flower epidermis of the adaxial surface are polygonal to irregular while the abaxial are polygonal to irregular. The anticlinal cell walls of the adaxial are slightly undulating while abaxial are undulating. Stomatas are scanty on abaxial surface and completely absent on adaxial surface (hypostomatic Table 2). Anisocytic stomata is abundant. Mature stomatas are Anomocytic, staurocytic, for adaxial surface only. The guard cell area was recorded in abaxial surface only (5  $\mu\text{m}$ , Table 2) while adaxial was absent. Abnormal stomata with aborted guard cell was observed (Plate 6E). Glandular trichomes are present on both surfaces but peltate trichome was found on adaxial surface only (Plate 6: B, F, N, Tab. 3). Crystals were common on both surfaces (Plate 6: D, G).

### 3.3 *H. rosa-sinensis* L.

Epidermal cells on the abaxial surface are pentagonal to polygonal while adaxial cells are pentagonal to polygonal to irregular with cuticular striations. Abaxial cells (65 x 39  $\mu\text{m}$ ) are smaller than adaxial cells (70 x 34  $\mu\text{m}$ ) as shown in Table 1. Anticlinal cell walls of abaxial surface are straight to curved, slightly undulate, while adaxial are slightly undulate. Trichomes on abaxial surface were glandular, two-armed trichome, four-armed, three-armed and five -armed non-glandular trichome on vein cells and only glandular on the adaxial surface (Table 3, Plate 4: E, H, J, K, L, M, R). Stomata distribution in the investigated taxa is amphistomatic (stomatas are abundant on both surfaces). Mature stomatas are anisocytic, staurocytic, anomocytic and brachyparacytic on both surfaces, while laterocytic stomatas were found on the adaxial surface only (Plate 4: A, B, C, F, N, P, Q, S). Guard cell area in adaxial (9  $\mu\text{m}$ ) is smaller than that of abaxial (12  $\mu\text{m}$ , Tab. 1). Abnormal stomata with parallel contiguous stomata was found in adaxial surfaces but aborted guard cell and

unopened stomatal pore on abaxial surface (Plate 4 B, D, G, I, J, T).

The flower epidermis of the adaxial surface are polygonal to irregular while that of abaxial surface are polygonal to irregular. The anticlinal cell walls of abaxial surface is sinuous to undulate while adaxial surface is slightly undulate. Stomatas are scanty on adaxial surface and abundant on the abaxial surface (hypoamphistomatic). Anisocytic, anomocytic and staurocytic are found on both surfaces. Staurocytic are abundant on both surfaces. (Plate

5F, G, J, L, M, O, P). The lowest guard cell was recorded in abaxial surfaces (8 μm) while adaxial had (9 μm, Tab. 2). Abnormal stomata with parallel contiguous stomata and one guard cell were found on the adaxial surface but only aborted guard cell were present on abaxial surface of the flower. (Plate 5 H, L, N). Three-armed, two-armed, unicellular, non-glandular and glandular trichomes were present. Non-glandular on abaxial surfaces but glandular on abaxial surface only (Plates 5 A, B, C, D, E, Table 3). Crystal druses are absent on both surfaces.

**Table 1. Epidermal feature of the leaves of *Hibiscus arnotianus*, *H. surattensis*, *H. rosa-sinensis* and *H. acetosella***

Species	Stomatal size (μm)		Epidermal cells (μm)		Guard Cells (μm)		Stomatal Index (%)		Stomatal Distribution	Epidermal Cell wall	
	Ad	Ab	Ad	Ab	Ad	Ab	Ad	Ab		Ad	Ab
	LxB	LxB	LxB	LxB							
<i>Hibiscus arnotianus</i>	30x20	35x20	40x26	56x23	5	4	7	43	hypoamphistomatic	Straight to Slightly undulate	Straight to undulate
<i>H. surattensis</i>	26x10	28x14	78x28	65x30	7	8	11	34	hypoamphistomatic	undulate	Straight to sinous
<i>H. acetosella</i>	32x18	29x15	75x34	59x24	14	5	22	38	amphistomatic	Slightly undulate	undulate
<i>H. rosa-sinensis</i>	26x17	34x19	70x34	65x39	9	12	44	39	amphistomatic	Slightly undulate	Slightly undulate

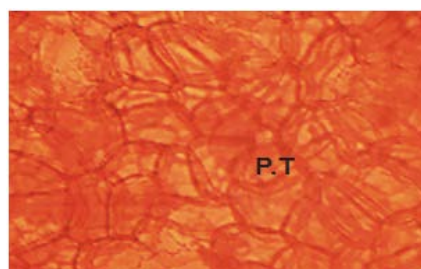
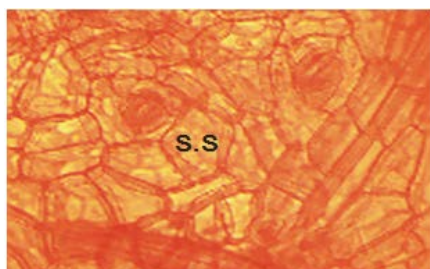
**Table 2. Epidermal feature of the flowers of *Hibiscus arnotianus*, *H. surattensis*, *H. rosa-sinensis* and *H. acetosella***

Species	Stomatal size (μm)		Epidermal cells (μm)		Guard Cells (μm)		Stomatal Index (%)		Stomatal Distribution	Epidermal Cell wall	
	Ad	Ab	Ad	Ab	Ad	Ab	Ad	Ab		Ad	Ab
	LxB	LxB	LxB	LxB							
<i>Hibiscus arnotianus</i>	39x26	32x22	88x17	76x26	5	7	13	4	amphistomatic	Slightly undulate	Sinous or undulate
<i>H. surattensis</i>	-	-	44x18	75x42	-	-	-	-	-	Straight to undulate	Slightly undulate
<i>H. acetosella</i>	-	27x20	35x25	57x20	-	5	-	25	amphistomatic	Slightly undulate	undulate
<i>H. rosa-sinensis</i>	26x15	27x16	36x16	65x29	9	8	25	4	hypoamphistomatic	Slightly undulate	Undulate or Sinous

**Table 3. Trichomes characteristic of leaves and flowers of *Hibiscus arnotianus*, *H. surattensis*, *H. rosa-sinensis* and *H. acetosella***

Species	Glandular trichomes Leaves		Non-glandular trichomes		Peltate Trichomes		Glandular Trichomes Flowers		Non-glandular Trichomes		Peltate Trichomes	
	Ad	Ab	Ad	Ab	Ad	Ab	Ad	Ab	Ad	Ab	Ad	Ab
	LxB	LxB	LxB	LxB	LxB	LxB	LxB	LxB	LxB	LxB	LxB	LxB
<i>Hibiscus arnotianus</i>	-	-	170x29	277x64	257x42	244x38	261x38	252x42	-	-	-	-
<i>H. surattensis</i>	237x42	262x62	289x40	181x38	-	-	-	262x32	-	-	-	-
<i>H. acetosella</i>	-	286x42	-	-	-	-	257x39	276x48	-	-	-	362x38
<i>H. rosa-sinensis</i>	316x44	197x42	253x40	-	-	-	-	147x43	278x62	279x38	-	-

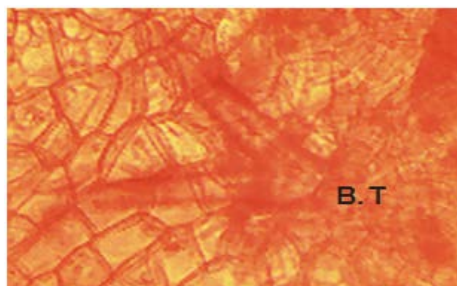
**Key:** Absent, Ad: Adaxial Surface (upper), Ab: Abaxial Surface (lower), L: Length, B: Breadth **Plate 1A – 1T.**



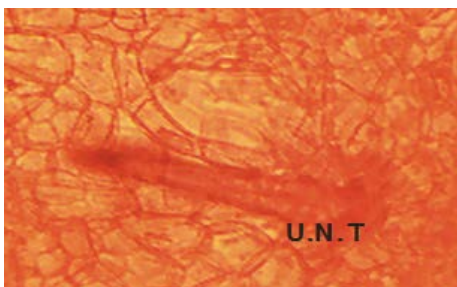
**Plate 1A.** A.S, Anisocytic and SS, staurocytic stomata of *H. arnotianus* (upper Surface) x 400

**Plate 1B.** P.T. Peltate trichome of *H. arnotianus* (upper Surface) x 400

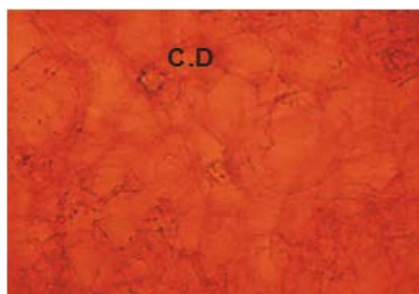




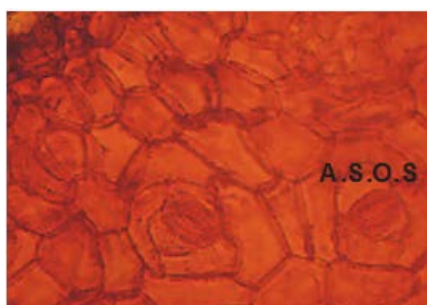
**Plate 1C.** B.T, Bifurcated (two-armed) trichome of *H. arnottianus* (upper Surface) x 400



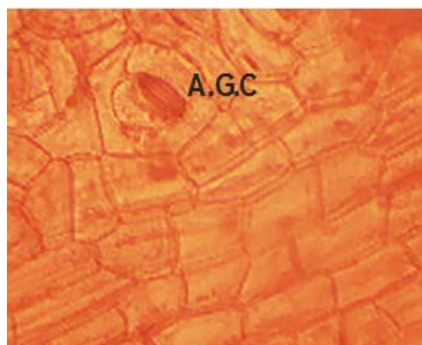
**Plate 1D.** U.N.T, Unicellular trichome of *H. arnottianus* (upper Surface) x 400



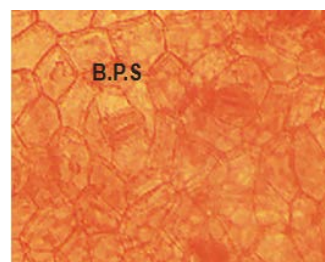
**Plate 1E.** C.D, Crystal druses of *H. arnottianus* (upper Surface) x 400



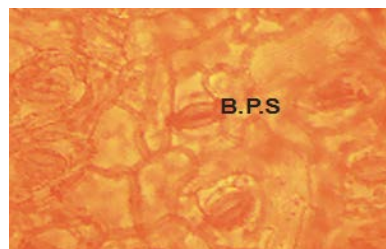
**Plate 1F.** A.S.O.S, Anomocytic stomata of *H. arnottianus* (upper Surface) x 400



**Plate 1G.** A.G.C, Aborted guard cell of *H. arnottianus* (upper Surface) x 400



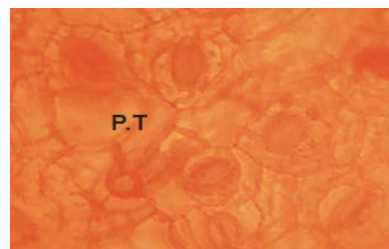
**Plate 1H.** B.P.S, Brachyparacytic of *H. arnottianus* (upper Surface) x 400



**Plate 1I.** B.P.S, Brachyparacytic of *H. arnottianus* (lower Surface) x 400



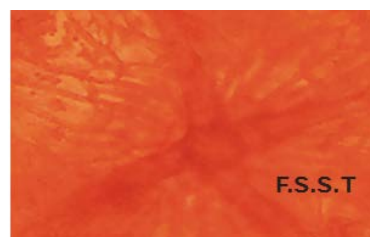
**Plate 1J.** A.S, Anisocytic of *H. arnottianus* (lower Surface) x 400



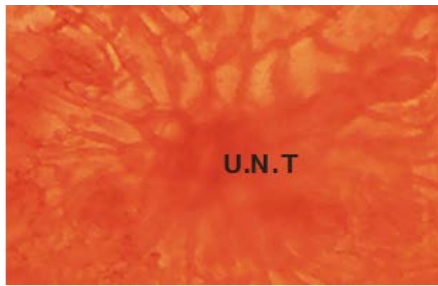
**Plate 1K.** P.T, Peltate of *H. arnottianus* (lower Surface) x 400



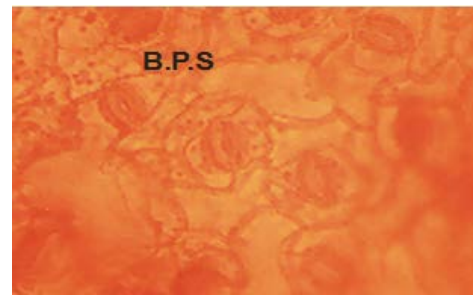
**Plate 1L.** U.S.T, Unopen stomatal pore of *H. arnottianus* (lower Surface) x 400



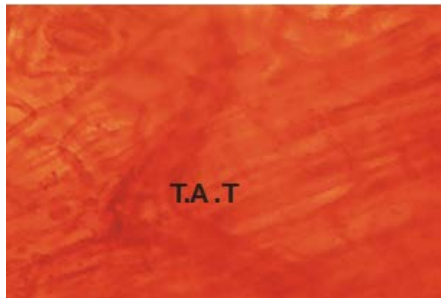
**Plate 1M.** U.S.T, Unopen stomatal pore of *H. arnottianus* (lower Surface) x 400



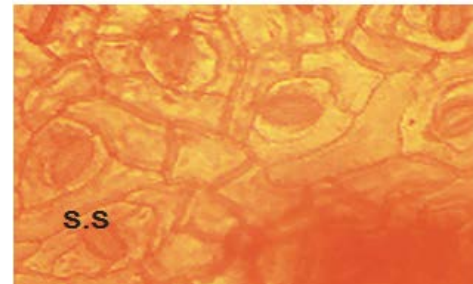
**Plate 1N.** U.N.T, Unicellular trichome of *H. arnottianus* (lower Surface) x 400



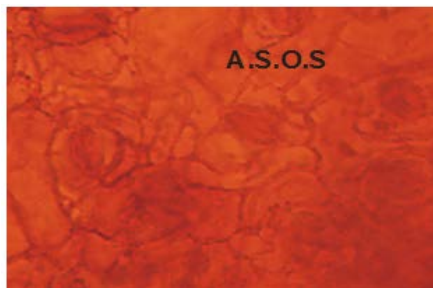
**Plate 1S.** B.P.S, Brachyparacytic stomata of *H. arnottianus* (lower Surface) x 400



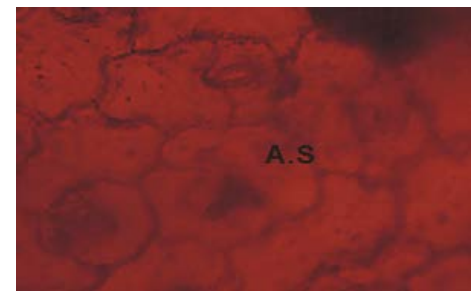
**Plate 1O.** T.A.T, Two-armed (bifurcated) trichome of *H. arnottianus* (lower Surface) x 400



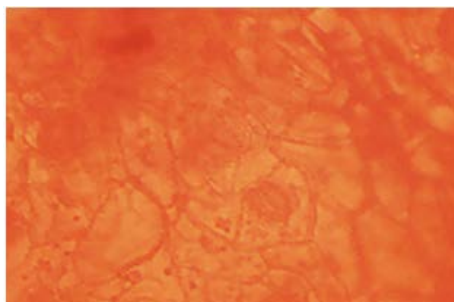
**Plate 1T.** S.S, Staurocytic stomata of *H. arnottianus* (lower Surface) x 400



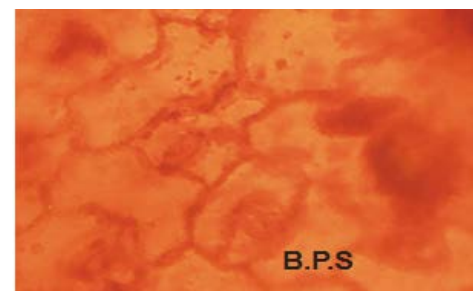
**Plate 1P.** A.S.O.S, Anomocytic stomata of *H. arnottianus* (lower Surface) x 400



**Plate 2A.** A.S, Anisocytic stomata of *H. surattensis* (lower Surface) x 400



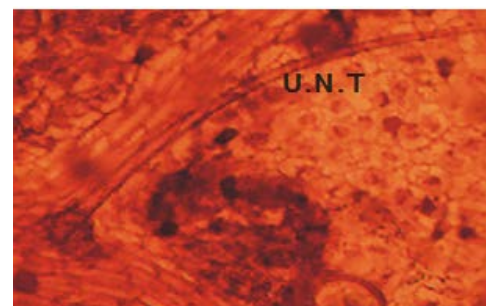
**Plate 1Q.** Two stomata sharing one subsidiary cell of *H. arnottianus* (lower Surface) x 400



**Plate 2B.** B.P.S, Brachyparacytic stomata of *H. surattensis* (lower Surface) x 400

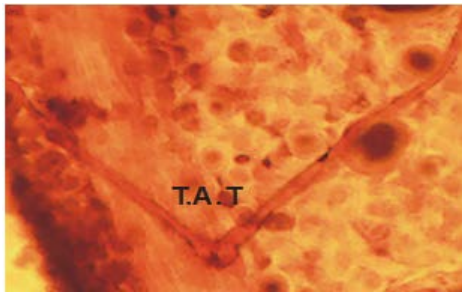


**Plate 1R.** A.G.C, Aborted guard cell of *H. arnottianus* (lower Surface) x 400

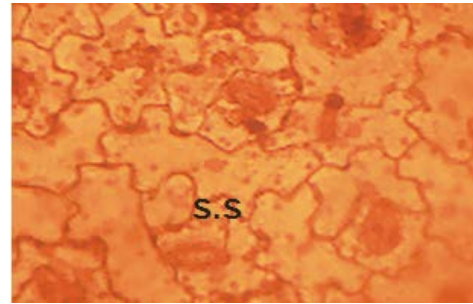


**Plate 2C.** U.N.T, Curved unicellular trichome of *H. surattensis* (lower Surface) x 100

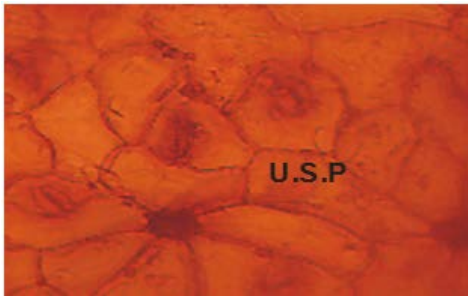




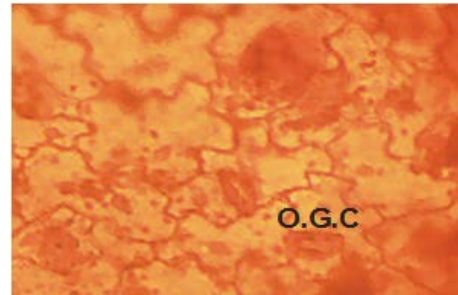
**Plate 2D.** T.A.T, Two-armed trichome of *H. surattensis* (lower Surface) x 100



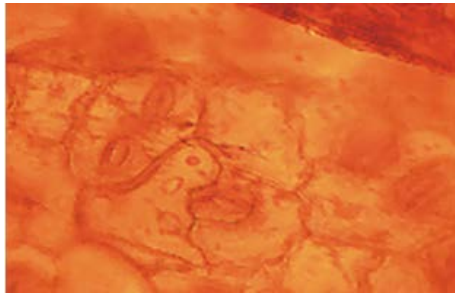
**Plate 2I.** S.S, Staurocytic stomata of *H. surattensis* (lower Surface) x 400



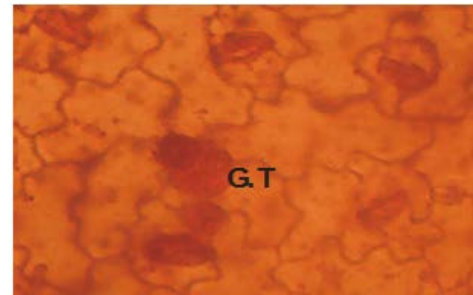
**Plate 2E.** U.S.P, Unopened stomatal pore of *H. surattensis* (lower Surface) x 400



**Plate 2J.** O.G.C, One guard cell and two stomata sharing one subsidiary cell of *H. surattensis* (lower Surface) x 400



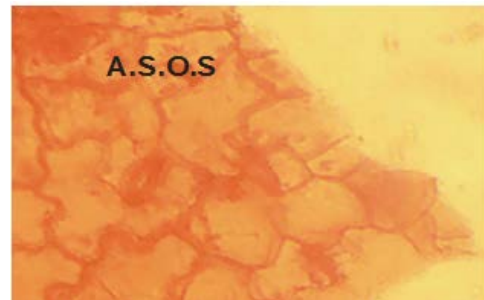
**Plate 2F.** Two stomata sharing one subsidiary cell of *H. surattensis* (lower Surface) x 400



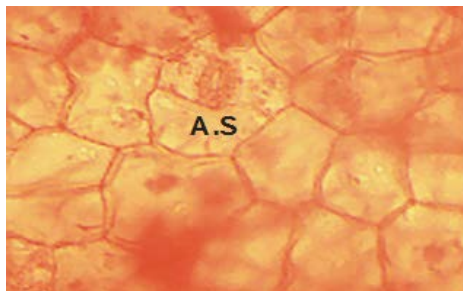
**Plate 2K.** G.T, Glandular trichome of *H. surattensis* (lower Surface) x 400



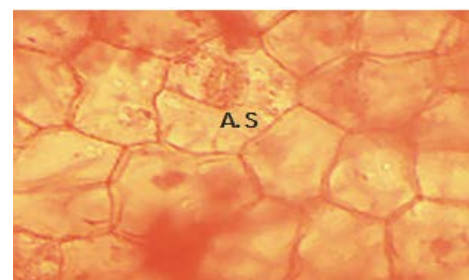
**Plate 2G.** A.G.C, Aborted guard cell of *H. surattensis* (lower Surface) x 400



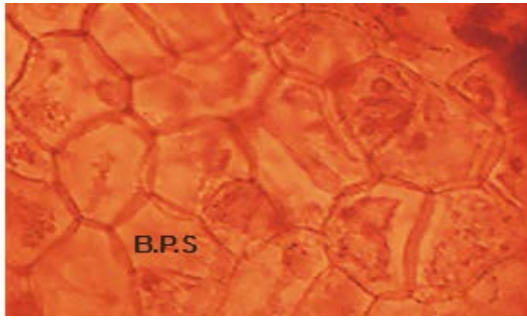
**Plate 2L.** A.S.O.S, Anomocytic stomata of *H. surattensis* (lower Surface) x 400



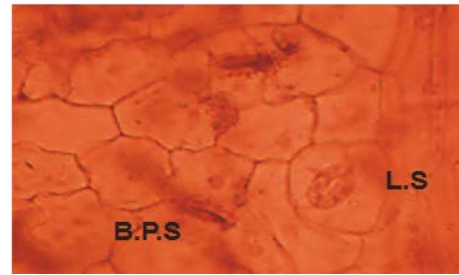
**Plate 2H.** A.S, Anisocytic stomata of *H. surattensis* (lower Surface) x 400



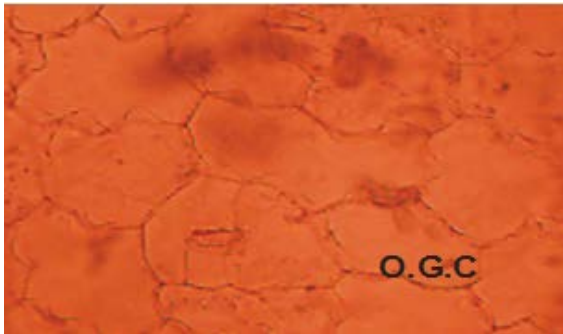
**Plate 2M.** A.S, Anisocytic stomata of *H. surattensis* (Upper Surface) x 400



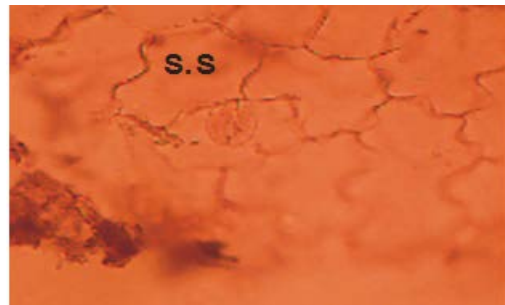
**Plate 2N.** B.P.S, Brachyparacytic stomata of *H. surattensis* (Upper Surface) x 400



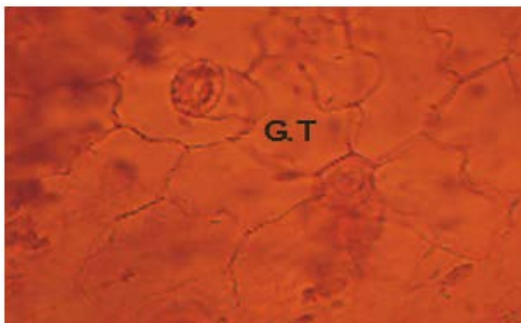
**Plate 2S.** B.P.S, Brachyparacytic and L.S, laterocytic stomata of *H. surattensis* (Upper Surface) x 400



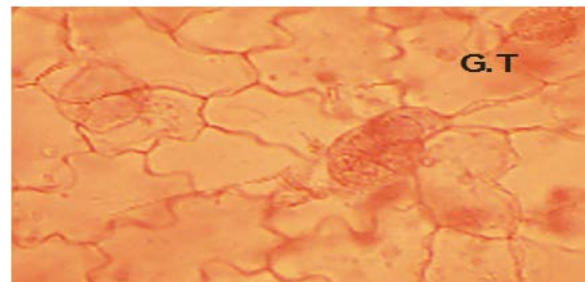
**Plate 2O.** O.G.C, One guard cell of *H. surattensis* (Upper Surface) x 400



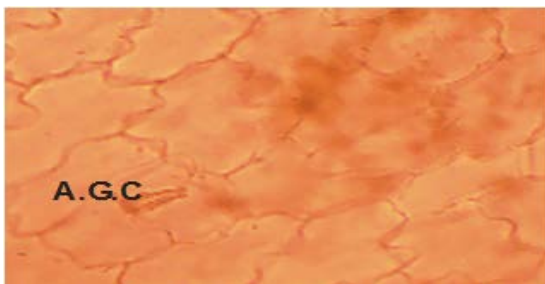
**Plate 2T.** S.S, Staurocytic stomata of *H. surattensis* (Upper Surface) x 400



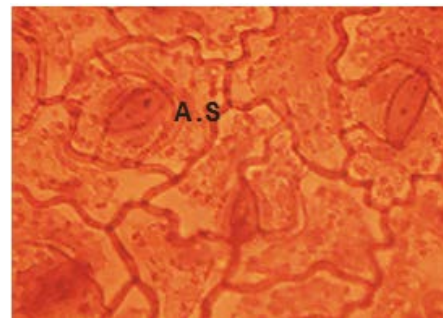
**Plate 2P.** G.T, Glandular trichome of *H. surattensis* (Upper Surface) x 400



**Plate 2U.** G.T, Glandular trichome of *H. surattensis* (Upper Surface) x 400



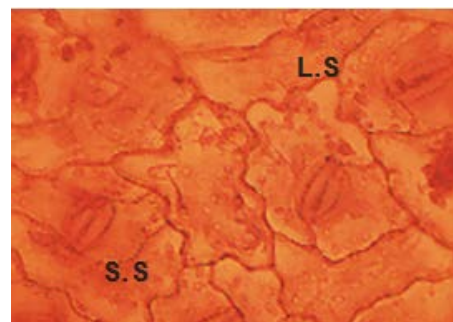
**Plate 2Q.** A.G.C, Aborted guard of *H. surattensis* (Upper Surface) x 400



**Plate 3A.** A.S, Anisocytic stomata of *H. acetosella* (Lower Surface) x 400

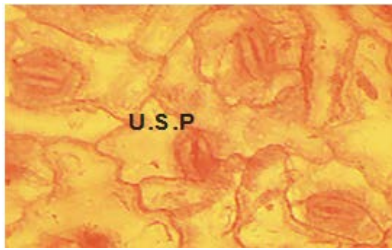


**Plate 2R.** U.S.P, Unopened stomatal pore of *H. surattensis* (Upper Surface) x 400

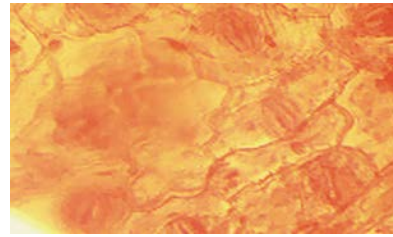


**Plate 3B.** L.S, Laterocytic stomata and S.S, staurocytic stomata of *H. acetosella* (Lower Surface) x 400

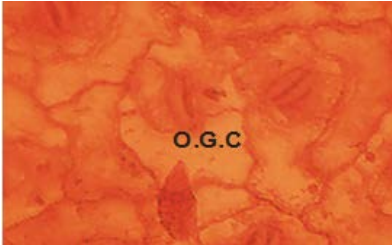




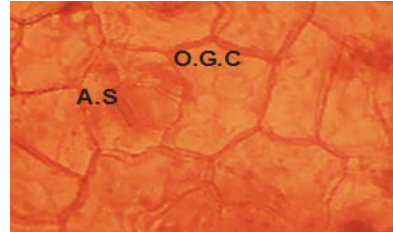
**Plate 3C.** U.S.P, Unopened stomata pore of *H. acetosella* (Lower Surface) x 400



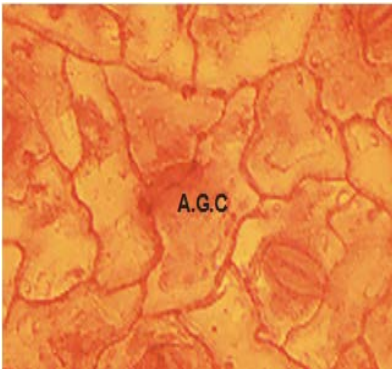
**Plate 3H.** Two stomata sharing one subsidiary cell of *H. acetosella* (Lower Surface) x 400



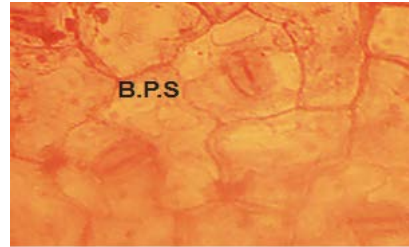
**Plate 3D.** O.G.C, One guard cell of *H. acetosella* (Lower Surface) x 400



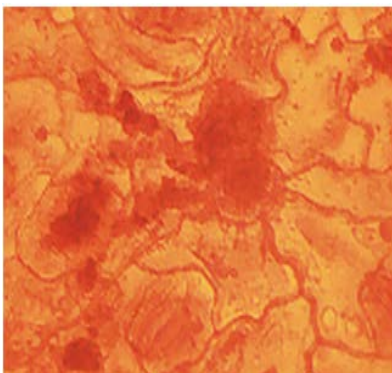
**Plate 3I.** O.G.C, One guard cell and A.S, anisocytic stomata of *H. acetosella* (upper Surface) x 400



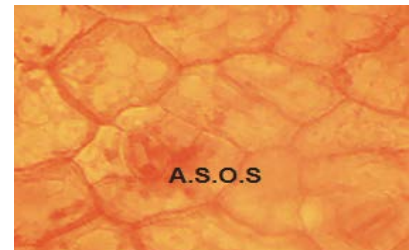
**Plate 3E.** A.G.C, Aborted stomata of *H. acetosella* (Lower Surface) x 400



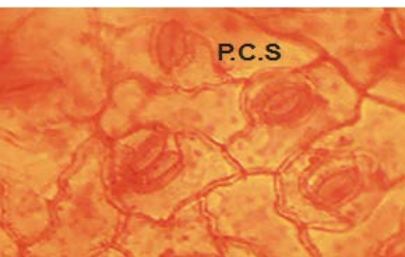
**Plate 3J.** B.P.S, Brachyparacytic stomata of *H. acetosella* (upper Surface) x 400



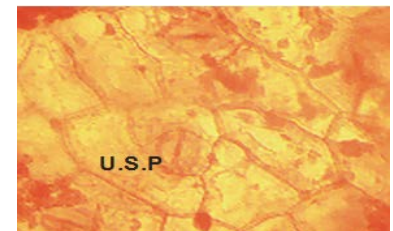
**Plate 3F.** G.T, Glandular trichome of *H. acetosella* (Lower Surface) x 400



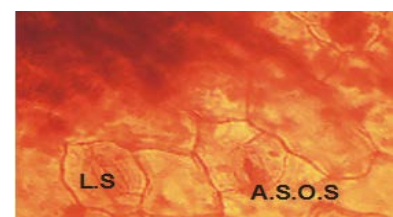
**Plate 3K.** A.S.O.S, Anomocytic stomata of *H. acetosella* (upper Surface) x 400



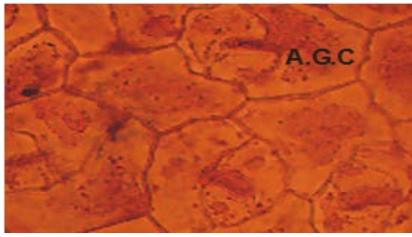
**Plate 3G.** P.C.S, Parallel contiguous stomata of *H. acetosella* (Lower Surface) x 400



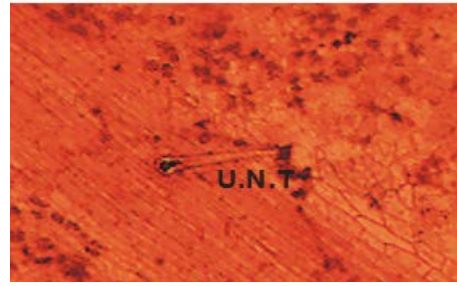
**Plate 3L.** U.S.P, Unopened stomata pore of *H. acetosella* (upper Surface) x 400



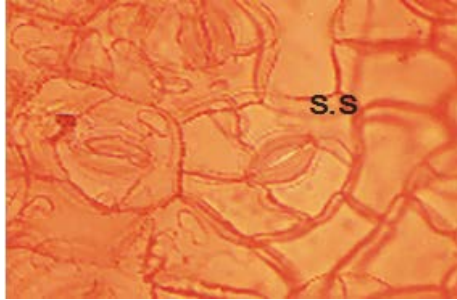
**Plate 3M.** L.S, Laterocytic stomata and A.S.O.S, Anomocytic stomata of *H. acetosella* (upper Surface) x 400



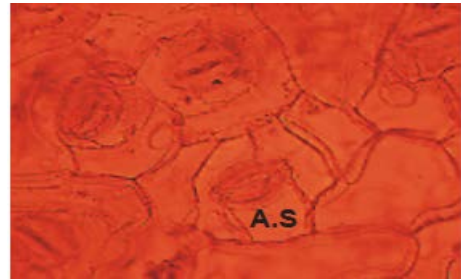
**Plate 3N.** A.G.C, Aborted guard cell of *H. acetosella* (upper Surface) x 400



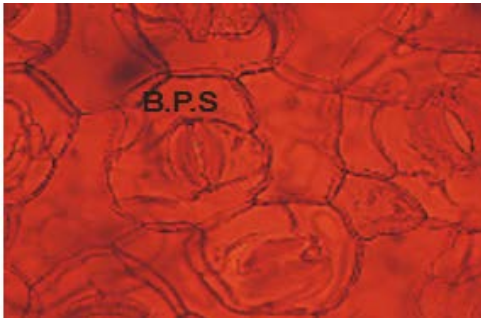
**Plate 4E.** U.N.T, Unicellular trichome on vein cells of *H. rosa-sinensis* (lower Surface) x 400



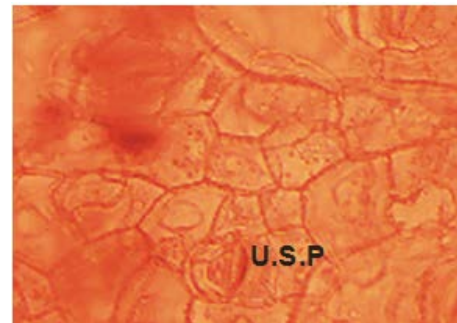
**Plate 4A.** S.S, Staurocytic stomata of *H. rosa-sinensis* (lower Surface) x 400



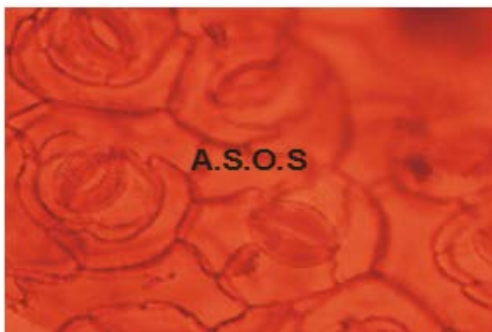
**Plate 4F.** A.S, Anisocytic stomata of *H. rosa-sinensis* (lower Surface) x 400



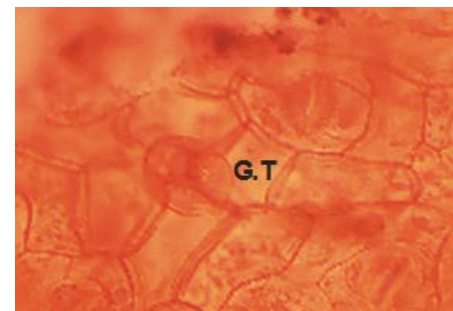
**Plate 4B.** B.P.S, Brachyparacytic stomata and one guard cell of *H. rosa-sinensis* (lower Surface) x 400



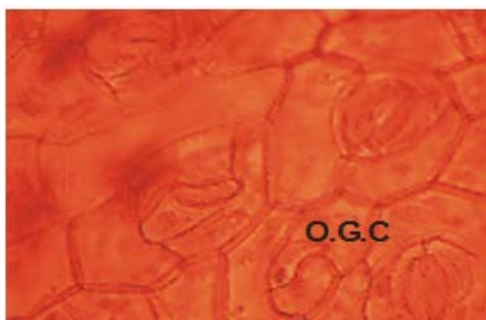
**Plate 4G.** U.S.P, Unopened stomata of *H. rosa-sinensis* (lower Surface) x 400



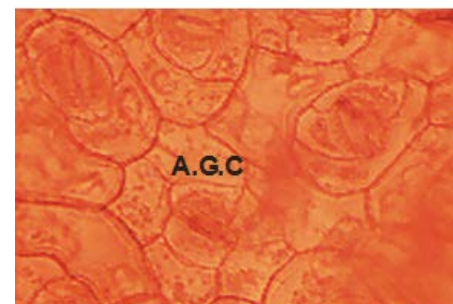
**Plate 4C.** A.S.O.S, Anomocytic stomata of *H. rosa-sinensis* (lower Surface) x 400



**Plate 4H.** G.T, Glandular trichome of *H. rosa-sinensis* (lower Surface) x 400



**Plate 4D.** O.G.C, One guard cell of *H. rosa-sinensis* (lower Surface) x 400

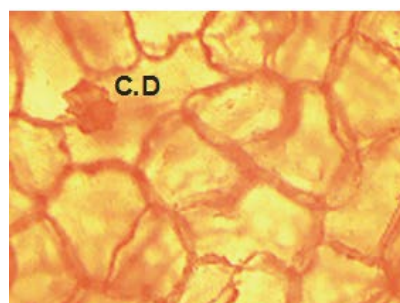


**Plate 4I.** A.G.C, Aborted stomata of *H. rosa-sinensis* (lower Surface) x 400

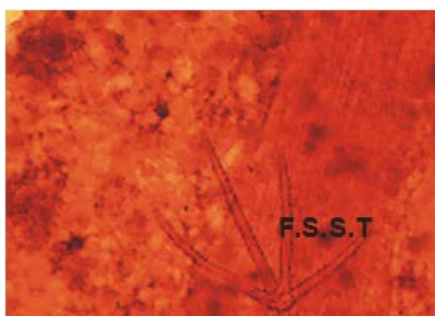




**Plate 4J.** T.A.T, Two-armed trichome of *H. rosa-sinensis* (lower Surface) x 400



**Plate 4O.** C.D, Crystal druses of *H. rosa-sinensis* (Upper Surface) x 400



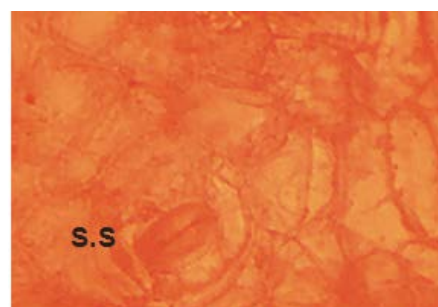
**Plate 4K.** F.S.S.T, Four-armed sessile stellate trichome of *H. rosa-sinensis* (lower Surface) x 200



**Plate 4P.** A.S.O.S, Anomocytic stomata of *H. rosa-sinensis* (Upper Surface) x 400



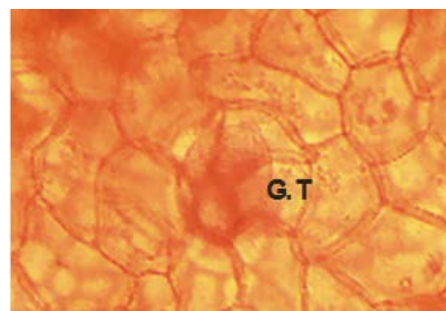
**Plate 4L.** T.H.A.T, Four-armed sessile stellate trichome of *H. rosa-sinensis* (lower Surface) x 400



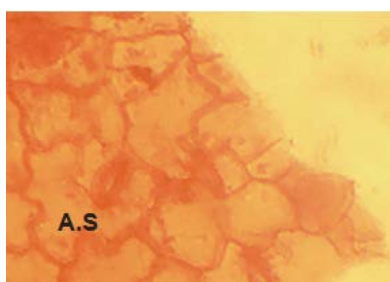
**Plate 4Q.** S.S, Staurocytic stomata of *H. rosa-sinensis* (Upper Surface) x 400



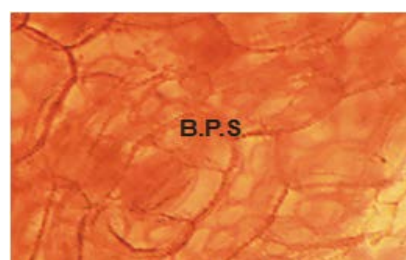
**Plate 4M.** F.I.S.S.T, FIVE-armed sessile stellate trichome of *H. rosa-sinensis* (lower Surface) x 100



**Plate 4R.** G.T, Glandular trichome of *H. rosa-sinensis* (Upper Surface) x 400

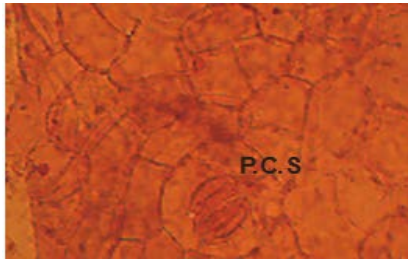


**Plate 4N.** A.S, Anisocytic stomata of *H. rosa-sinensis* (Upper Surface) x 400

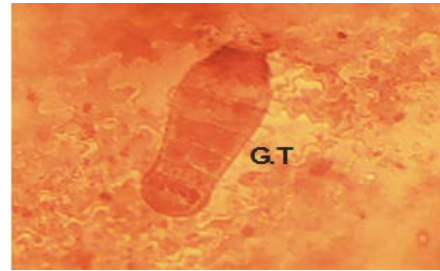


**Plate 4S.** B.P.S, Brachyparacytic stomata of *H. rosa-sinensis* (Upper Surface) x 400





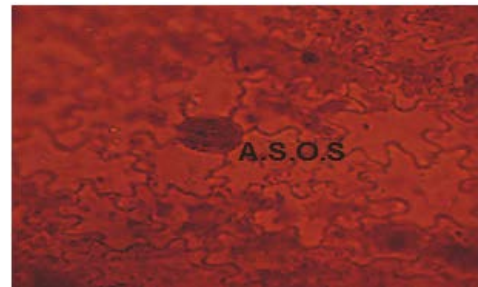
**Plate 4T.** P.C.S, Parallel contiguous stomata of *H. rosa-sinensis* (Upper Surface) x 400



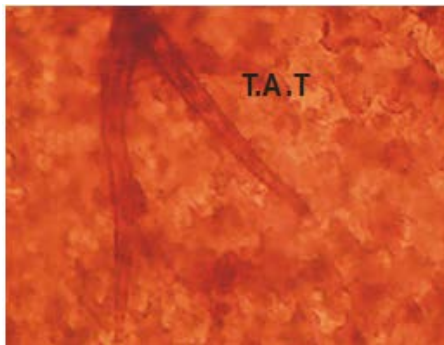
**Plate 5E.** G.T, Glandular trichome of *H. rosa-sinensis* (lower Surface) x 400



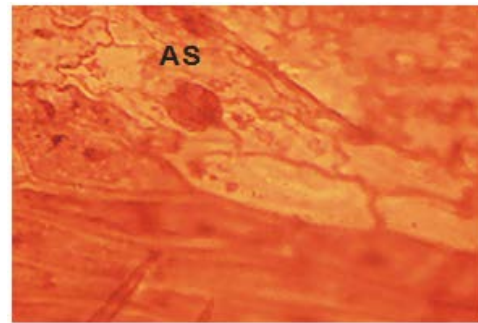
**Plate 5A.** T.H.A.T, Three-armed sessile stellate trichome of *H. rosa-sinensis* (lower Surface) x 400



**Plate 5F.** A.S.O.S, Anomocytic stomata of *H. rosa-sinensis* (lower Surface) x 400



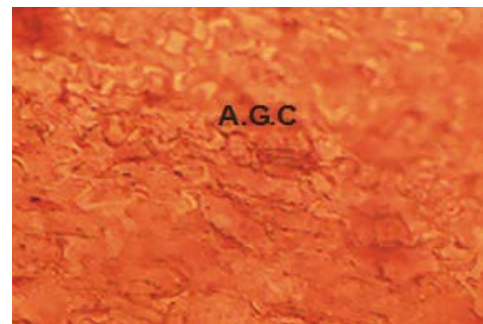
**Plate 5B.** T.A.T, Two-armed trichome (Bifurcated) of *H. rosa-sinensis* (lower Surface) x 400



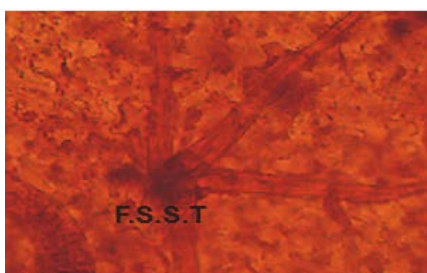
**Plate 5G.** A.S, Anomocytic stomata of *H. rosa-sinensis* (lower Surface) x 400



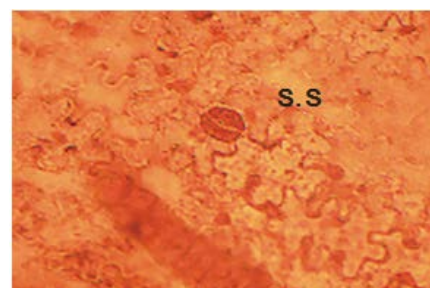
**Plate 5C.** U.N.T, Unicellular trichome of *H. rosa-sinensis* (lower Surface) x 100



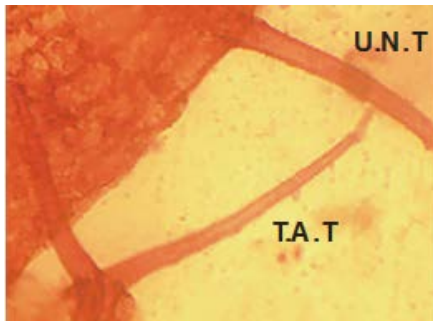
**Plate 5H.** A.G.C, Aborted guard cell of *H. rosa-sinensis* (lower Surface) x 400



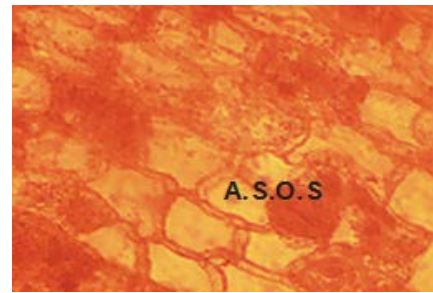
**Plate 5D.** F.S.S.T, Four-armed sessile stellate trichome of *H. rosa-sinensis* (lower Surface) x 400



**Plate 5I.** S.S, Staurocytic stomata of *H. rosa-sinensis* (lower Surface) x 400



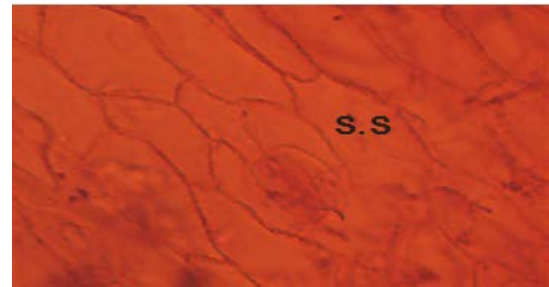
**Plate 5J.** T.A.T.T, Two-armed trichome (Bifurcated) and unicellular trichome of *H. rosa-sinensis* (lower Surface) x 400



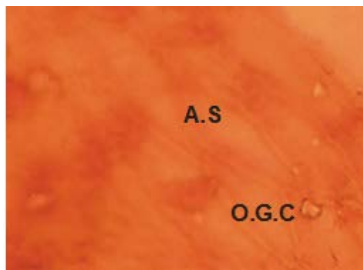
**Plate 5O.** A.S.O.S, Anomocytic stomata of *H. rosa-sinensis* (Upper Surface) x 400



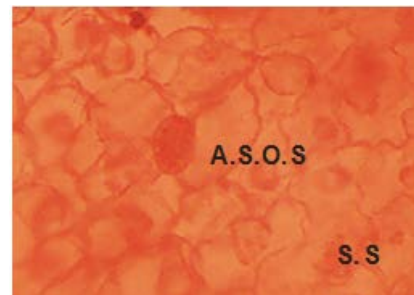
**Plate 5K.** G.T, Glandular trichome of *H. rosa-sinensis* (Upper Surface) x 400



**Plate 5P.** S.S, Staurocytic stomata of *H. rosa-sinensis* (Upper Surface) x 400



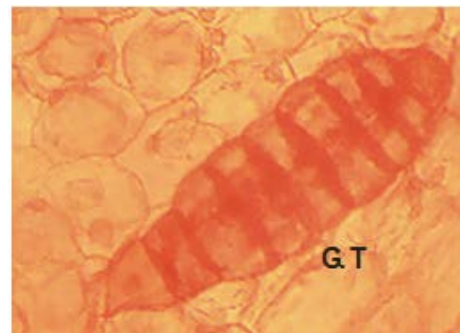
**Plate 5L.** A.S, Anisocytic stomata and O.G.C, one guard cell of *H. rosa-sinensis* (Upper Surface) x 400



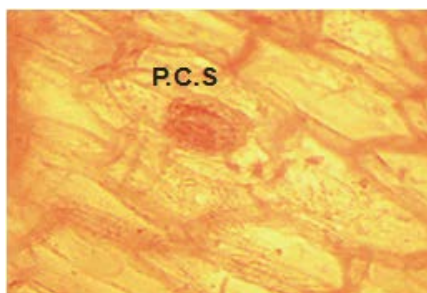
**Plate 6A.** A.S.O.S, Anomocytic and S.S, Staurocytic stomata of *H. acetosella* (Lower Surface) x 400



**Plate 5M.** S.S, Staurocytic stomata of *H. rosa-sinensis* (Upper Surface) x 400



**Plate 6B.** G.T, Glandular trichome of *H. acetosella* (Lower Surface) x 400

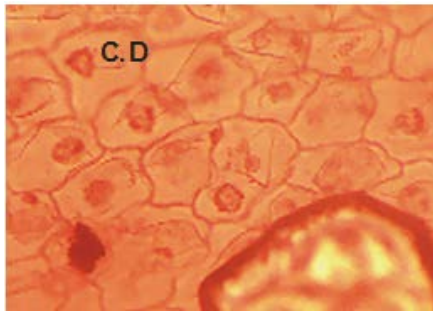


**Plate 5N.** P.C.S, Parallel contiguous stomata of *H. rosa-sinensis* (Upper Surface) x 400

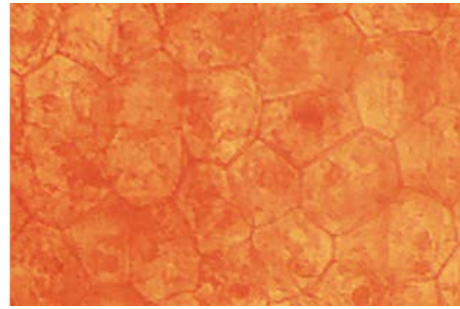


**Plate 6C.** A.S.O.S, Anomocytic stomata of *H. acetosella* (Lower Surface) x 400





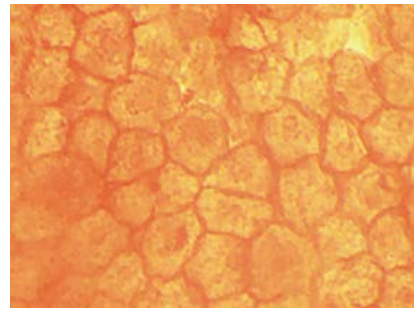
**Plate 6D.** C.D, Crystal druses of *H. acetosella* (Lower Surface) x 400



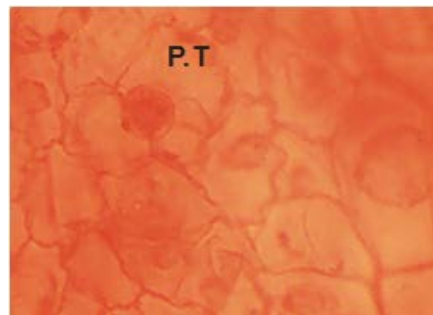
**Plate 6I.** E.P, Epidermal cell of *H. acetosella* (Upper Surface) x 400



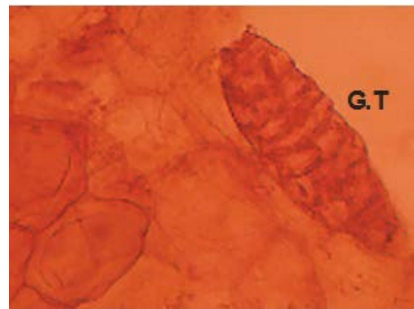
**Plate 6E.** S.S, Staurocytic ad aborted stomata of *H. acetosella* (Lower Surface) x 400



**Plate 7A.** E.P, Epidermal cell of *H. acetosella* (Upper Surface) x 400



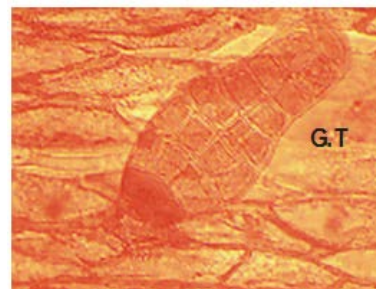
**Plate 6F.** P.T, Peltate trichome of *H. acetosella* (Lower Surface) x 400



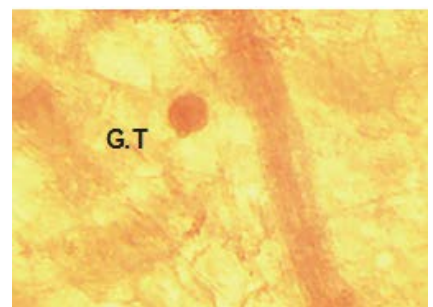
**Plate 7B.** G.T, Glandular trichome of *H. acetosella* (Lower Surface) x 400



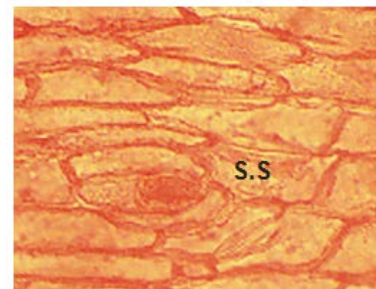
**Plate 6G.** C.D, Crystal druses of *H. acetosella* (Upper Surface) x 400



**Plate 8A.** G.T, Glandular trichome of *H. arnottianus* (Upper Surface) x 400

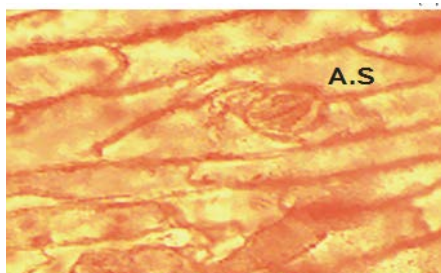


**Plate 6H.** G.T, Glandular trichome of *H. acetosella* (Upper Surface) x 400



**Plate 8B.** S.S, Staurocytic stomata of *H. arnottianus* (Upper Surface) x 400

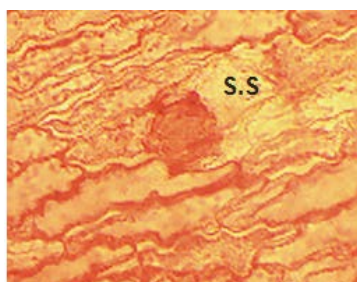




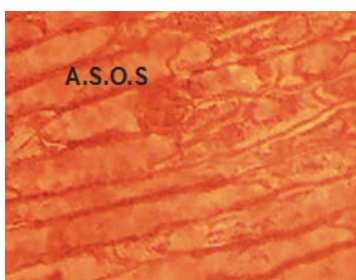
**Plate 8C.** E.P, Epidermal cell and A.S, Anisocytic stomata of *H. arnottianus* (Upper Surface) x 400



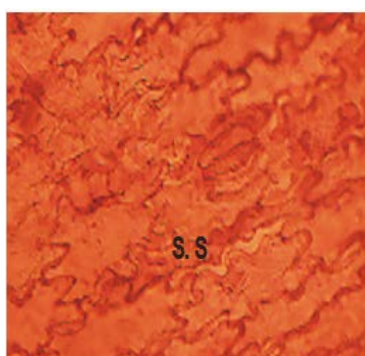
**Plate 8H.** P.C.S, Parallel contiguous of *H. arnottianus* (Lower Surface) x 400



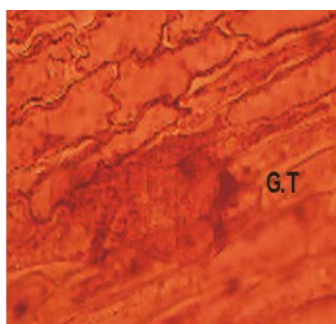
**Plate 8D.** S.S, Staurocytic stomata of *H. arnottianus* (Lower Surface) x 400



**Plate 8E.** A.S.O.S, Anomocytic stomata of *H. arnottianus* (Lower Surface) x 400



**Plate 8F.** S.S, Staurocytic stomata of *H. arnottianus* (Lower Surface) x 400



**Plate 8G.** G.T, Glandular trichome of *H. arnottianus* (Lower Surface) x 400

## 4. Discussion

Anatomical features are widely used in systematic for identification, for placing anomalous group in satisfactory position in classification and for indicating patterns of relationship that may have been obscured by superficial convergence in anatomical features and morphology [29].

Anatomical evidence have proved useful in characterization of *H. surattensis*, *H. arnottianus*, *H. acetosella* and *H. rosa-sinensis* of the leaves and flowers respectively. Epidermis was pentangular to polygonal to irregular in shape and size with slightly undulating, undulate and sinous anticlinal walls. In *H. surattensis*, stomatas are absent on abaxial and adaxial surfaces of flower but present in both surfaces of the leaves (Hypostomatic) and mostly anisocytic with some brachyparacytic and staurocytic types. In *H. arnottianus*, stomatas are present on both surfaces of the leaves and flowers but scanty on the abaxial surface then abundance on adaxial surface (Hypoamphistomatic). On the species of *Talinum*, Essiett and Akpabio [30] shows that, it is possible for most species to have more than one type of stomata. The present of various types of stomata in the taxa is of taxonomic interest in this study, because it can distinguish *H. acetosella* as having laterocytic stomata on both surfaces of the leaves and present on the adaxial surface of *H. surattensis* only, while staurocytic stomata is abundantly present on the abaxial surface of *H. rosa-sinensis* flower but in addition, brachyparacytic stomata occurred on *H. arnottianus* leaves, both adaxial and abaxial surfaces but in flower, brachyparacytic are absent in all surfaces.

Another interesting character is the presence of abnormal stomata with one guard cell, an aborted guard cell, parallel contiguous stomata are present on abaxial surface of *H. acetosella* and adaxial surface of *H. rosa-sinensis*, two stomatas sharing one subsidiary cell and guard cells respectively. There is no evidence to suggest that all the abnormalities are the result of environmental factors which were confirmed by Dehnel [31]. The presence and combination of different types of stomata on the epidermal surfaces highlighted by Metcalfe and Chalk [32] can be useful in the classification and delimitation of the taxa they studied. Some attempts have been made to use stomatal characters as an aid to classification. The diversity of the stomata is now reported in a number of plates and example in *Phyla nodiflora*, *Convolvulaceae* and *Asteraceae* [33]. The function of stomata is associated with various physiological processes and with survival of each individual plant [29]. It is expected that stomatal

structure will be under strong and highly integrated genetic control and that modification of stomata during evolution will reflect both general relationship and evolutionary trend. The guard cell area, stomatal index provides value that would serve as parameters for comparison among taxa.

The stomatal index is independent of the environment size or portion of the leaf surface. Size of the intervening epidermal cells are reliable factors for identification [32]. Trichomes were present on vein cells. Glandular trichome on the adaxial surface of *H. surattensis* leaves, abaxial surface of *H. acetosella* and on both surfaces of *H. rosa-sinensis*, present on abaxial surface of *H. surattensis* and it was totally absent on the adaxial surface of *H. surattensis* flower and non-glandular trichome on both surface of *H. arnottianus* and *H. surattensis* leaves but absent on both surfaces of the flower. Peltate trichomes are found on both surfaces of *H. arnottianus*. The high density of the long unicellular hairs in them also serve to reduce the rate of transpiration in the plants. The importance of trichomes in taxonomy has been highlighted by Stace [34] in the family *Combretaceae*, Illoh and Inyang [35].

Stomatal index varied between the four species of *Hibiscus* leaves on both adaxial and abaxial surfaces. The highest stomatal index was found on the leaf of *H. rosa-sinensis* on the adaxial surface (44%). It is useful for the separation of *H. surattensis* flower (Table 3) in line with the findings of Isawumi [36] and Abdulrahman and Oladele [37]. Stomatal index is independent on the environmental size of the leaf surface of the epidermal cell and it is a reliable factor for identification. The function of stomata was associated with various physiological process and success of each individual plant (Essiett, 2004). The guard cell area and stomatal index provides values that would serve as parameters for comparison among taxa. The importance of crystals in taxonomy as diagnostic tools was emphasized by Amos [38], he went further to mention that their mode of distribution is equally important in taxonomy. The presence of different types of crystals of calcium carbonate known as druses of crystals and their distribution in the leaves and flowers are therefore of interest. This was conflicted with Dehnel [31].

## 5. Conclusion

In conclusion, the analysis of these research works of *H. arnottianus*, *H. surattensis*, *H. rosa-sinensis* and *H. acetosella* have been distinguished on the basis of microscopy evaluation. The presence of different types of stomatas on the leaves and flowers of *Hibiscus* species studied shows that these bases provide some justification for the description, identification and collection of plant species.

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