

Leaf epidermal studies of three species of *Acalypha* Linn. (Euphorbiaceae)

^{1*}Essiett Uduak Aniesua and ¹Etukudo Inyene Silas

Department of Botany and Ecological Studies, University of Uyo, Uyo

ABSTRACT

Leaf epidermal studies of three species of *Acalypha* are described. The mature stomata were laterocytic, staurocytic, anisocytic, paracytic and diacytic. The abnormalities noticed include unopen stomatal pore, two stomata sharing one subsidiary cell, one guard cell, parallel contiguous and aborted guard cell. *A. godseffiana* can be distinguished by parallel contiguous on both surfaces. Curved uniseriate non-glandular trichomes were restricted to *A. wilkesiana*. Two stomata sharing one subsidiary cell occurred only on the lower surface of *A. hispida*. The shapes of epidermal anticlinal cell walls, guard cell areas, stomata index and trichomes varied. The differences are of taxonomic importance and can be used to identify and delimit each species by supporting other systematic lines of evidence.

Keywords: *Acalypha* species, Epidermal, Stomata, Nigeria, Euphorbiaceae.

INTRODUCTION

Euphorbiaceae, the spurge family of the flowering plants with 500 genera and around 7,500 species. Most are herbs, but some especially in the tropics are also shrubs and trees some are succulent and resemble cacti (Davis *et al.*, 2007). *Acalypha* Linn. is a plant genus of the family Euphorbiaceae. It is the sole genus of the subtribe Acalyphinae with about 450 to 500 species of herbs and shrubs. *Acalypha hispida* Burm F. (Chenille Plant) is a flowering shrub, it is cultivated as houseplant because of its colourful and texturally exciting flowers, it is by far the best known species, others are grown for foliage and a number of cultivars have been developed. Its common names include chenille plant, philippine medusa, red-hot cat tail "fox tail" (Grubben and Denton, 2004). The chenille plant is a vigorous upright, coarse textured shrub in habit that usually attains a height of 5 to 6ft and spreads 6 to 8ft, it is moderate in density and has a fast growth rate. The leaves are ovate in shape, medium green leaves which alternates in arrangements, it has a serrated margin, it is pinnate in venation and dark green in colour. Fruits are inconspicuous (Sagun *et al.*, 2010).

Acalypha wilkesiana Muell Arg. Its common names include copper leaf, Joseph's coat, fire dragon, beef steak plant and match-me-if you can (Christman, 2004). It is a native to Fiji and nearby Islands in the southern pacific and it is a popular outdoor plants that provides colour through out the year, although it is also grown indoor as a container plant (Gilman, 1999; Christman, 2004). *Acalypha wilkesiana* are succulent with sappy stalks which tends to loose sappiness with age, it is round upright in habit, dense and coarse in texture, has a fast growth rate.

Leaf arrangement is alternate it has a simple leaf type and dentate leaf margin, it is reniform in shape, and pinnate in venation. Leaf type and persistent is evergreen (Sagun *et al.*, 2010). *Acalypha godseffiana* Var: are mainly shrub, its common names is *acalypha* green frills. It has a narrow dropping green leaves with creamy white margins (Gilman, 1999; Christman, 2004).

The plant is known for its grossy white margined green leaves. They produce narrow pendant leaves that have white/pale yellow margin. This upright rounded shrub produce twisted leaves forming a compact rounded habit and

produces green foliage. Leaves are simple in shape and are arranged alternately opposite, it may have stipules, i.e. possess a serrated margin to crenate teeth 1.2 by 2.3mm, with a gland on tooth tip (Sagun *et al.*, 2010).

The species of *Acalypha* are prominent in the traditional medicinal practice of most tribes in Africa and Asia (Duraipandiyar *et al.*, 2006; Sofowora, 1982). *Acalypha wilkesiana* is used in West Africa for the treatment of headache and colds in Nigeria, the cold extracts of the leaves is used to bath babies with skin infection (Adesina *et al.*, 2000). The leaf poultice is deemed good for headache, swellings and cold in Trinidad. Its leaf extract is active against Gram +ve bacteria, the extracts of seed have immunomodulating properties that work against some tumors (Bussing *et al.* 1999). Cooked leaves of some other species of *Acalypha* are used to relieve post partum pains and a root decoction as a laxative. It has also been reported to be used for cutaneous and subcutaneous parasitic infections (Sabrina *et al.* 2005). The leaves are also compounded with the leaves of other drug plants into a drug for children with rabies in Southern Nigeria. *Acalypha wilkesiana* and *Acalypha godseffiana* has antimicrobial properties (Ogundaini, 2005). According to Ogundaini (2005), the expressed juice or boiled decoction is used for the treatment of gastrointestinal disorders and fungal skin infections such as *Pityriasis versicolor*, *Impetigo contagiosa*, *Candida intertrigo*, *Tinea versicolor*, *Tinea corporis* and *Tinea pedis*. In southern Nigeria, the leaves of these plants are eaten as vegetables in the management of hypertension, consequent upon which we had earlier monitored the effect of the plants leaves of normal rabbits (Ikewuchi *et al.*, 2008). *Acalypha hispida* has medicinal value in south Eastern Asia (Soladoyei *et al.*, 2008).

The aim of this work is to distinguish some *Acalypha* species existing in Nigeria by stomatal and trichomes types and the significance of the study is to contribute useful new characters to assess the identification and taxonomic position of species in the future studies of *Acalypha* species. Thus, providing a useful tool for collection and preservation of these species. Leaf venation by Levin (1986 a,b,c) Sehegal and Paliwal (1974), Hickey and Wolfe (1975). Trichomes by Inamdar and Gangadhara (1977), Cutler (1968) Kakkar and Paliwal (1972).

Foliar epidermal feature of some Euphorbiaceous taxa have also been investigated (Aworinde *et al.*, 2008; Laura *et al.*, 2008 and Thakur and Patil, 2008). Aworinde *et al.* (2009) used taxonomic significance to distinguish *Acalypha* members in Nigeria which had leaf epidermal characters such as pattern of epidermal cells, types of stomata and presence of trichomes are constant in some species and variable in other and thus are of great significance in understanding the relationships between and within species.

The methods of numerical taxonomy have been used by many authors in classifying plants as well as interpreting results of taxonomic studies (Fabio, 2007; Borazan and Babac, 2003) methods are considered to be unbiased indicators of the similarity of difference between the taxa, which are in turn used to arrange taxa in hierarchical order (Agbagwa, and Okoli, 2005; Quike, 1993).

Kadiri *et al.* (2009) carried out a comparative foliar epidermal characteristics of 15 species of *Acalypha* L. in West Africa are analysed using both light and scanning electron microscopy presence of hypostomatic leaves supports the series accepted by Bethem and Hooker. In taxa of other series amphistomatic leaves were recorded. Among other taxonomically useful features of the genus are polygonal and irregular epidermal cell shaped, curved and undulated anticlinal walls, presence of regular to irregular striae and deposition of waxy flakes on the leaf surfaces.

MATERIALS AND METHODS

The fresh leaves of *Acalypha hispida* Burm. F was collected from a flower bed close to the convocation park at University of Uyo main campus while the fresh leaves *Acalypha wilkesiana* Muell Arg. And *Acalypha godseffiana* Var. were collected behind Botany and Ecological Studies laboratory I at University of Uyo main campus. Akwa Ibom State.

Microscopic Examination

Epidermal peels of both abaxial and adaxial surfaces were made by placing the leaf blade taken from a standard median portions of the leaves on a clean glass slab; with the surfaces to be studied facing downward. The specimens were irrigated with water holding it downwards from one end. The epidermis above the desired surfaces was scrapped of carefully with a sharp razor blade loose cells were washed away from the epidermal peel with the aid of soft camel hairbrush and water until the desired epidermis below was reached.

The epidermal peels were stained with 1% aqueous solution of safranin O for 4-8 minutes, then rinsed carefully in water to remove excess stain and mounted in 10% glycerol. Guard cell area was calculated by Franco's constant method 0.7854 (Guard cells area = length x width x 0.7854). The stomatal index was determined according to Metcalfe and Chalk (1979). Using the formula:

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

Where

S - Number of stomata per unit area

E - Number of epidermal cells in the same area.

Specimens were observed at X400 objective magnification. All measurement 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 with a Motic Microscope version 2.0ml (Essiett and Akpabio, 2009; Essiett *et al.*, 2010; Abdulrahman and Oladele, 2003).

RESULTS

Salient features of the epidermal morphology of 3 species of *Acalypha* are summarized in Table 1. The most important characters are being described in some details below.

Acalypha hispida Burm. F

Epidermal cells on the abaxial and adaxial epidermis are mainly polygonal and irregular, variable in size and shape. Abaxial cells (29.1x18.6µm) are larger than the adaxial cells. (26.4 X 15.5µm) as shown in Table 1. Anticlinal cell walls of abaxial surface are undulate while the adaxial surface are straight to slightly undulating.

Few long, short pointed to curved apices unicellular non-glandular trichome are found on the vein cell and in the surrounding epidermal cell of both surfaces of lamina. The distribution of stomata in the investigation is hypoamphistomatic (stomata abundant on the abaxial surface and scanty on the adaxial epidermis). Four stomatal types are found mostly laterocytic, staurocytic, anisocytic and paracytic, but only laterocytic on adaxial surface randomly distributed and their axes is oriented in different directions. Guard cell areas on abaxial surfaces (average 42.1µm²) are larger than adaxial surface (average 41.7µm²). Abnormal stomata with aborted guard cell, one (single) guard cell and two stomata sharing one subsidiary cell are present (Plate 1A-I).

Acalypha wilkesiana Muell Arg.

Epidermal cell on the abaxial and adaxial surfaces are mainly polygonal and irregular variable in size and shape. Anticlinal cell walls of abaxial surface is highly undulate while those on the adaxial surface is straight to slightly undulating. Abaxial cell (22.3 x 18.3µm) are larger in size than the adaxial cells (20.9 x 16.9µm) as shown in Table1.

Few long, short pointed to curved apices uniseriate non-glandular trichomes are found on the vein cells of both abaxial and adaxial surfaces three stomatal types are found such as laterocytic, staurocytic and anisocytic laterocytic occur on both surfaces and it is found to be abundant on both surfaces. The distribution is hypoamphistomatic (stomata abundant on abaxial surface and scanty on adaxial surface).

Guard cell areas on adaxial surface (average 41.9 µm²) are larger than the abaxial surface (average 39.5µm²). Abnormal stomata with one guard cell were observed in the abaxial surface only (Plate 3A-F).

Acalypha godseffiana Var.

Epidermal cells on the abaxial and adaxial epidermis are mainly polygonal and irregular variable in size and shape. Abaxial cells (24.3 x 19.4 µm) are larger in size than the adaxial cells (22.6x14.8 µm) as shown in Table 1. Anticlinal cell walls of abaxial surface is undulate while those on the adaxial surface are straight to slightly undulating.

Few curved apices unicellular non-glandular trichome are found on the vein cells and surrounding epidermal cells of abaxial surface. The distribution of stomata in these investigation taxa is hypoamphistomatic (abundant on abaxial surface and scanty on adaxial surface).

Stomata present, although more numerous on the abaxial epidermis. Five stomatal types are found mostly laterocytic, staurocytic, anisocytic, paracytic and diacytic, but only laterocytic occurs on both abaxial and adaxial surfaces randomly distributed and their axes are oriented in different directions, laterocytic is the dominant stomata present. Guard cell area on abaxial surface (average 44.1µm²) is larger than the adaxial surface (average 40.4 µm²). Abnormal stomata with parallel contiguous, aborted guard cell and unopen stomatal pore were observed on the abaxial surface while parallel contiguous stomata was also observed in the adaxial surface (Plate 2A-L).

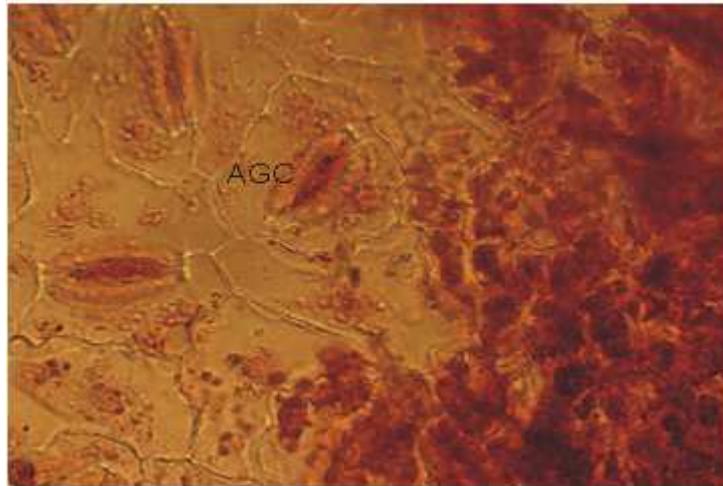


Plate .1A, A.G.C: Aborted guard cell of *Acalypha hispida* (lower surface)x400

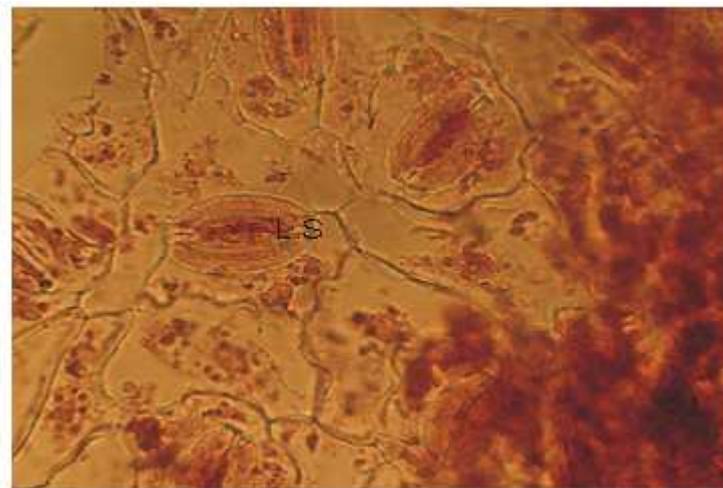


Plate .1B, L.S: Laterocytic Stomata of *Acalypha hispida* (lower surface)x400

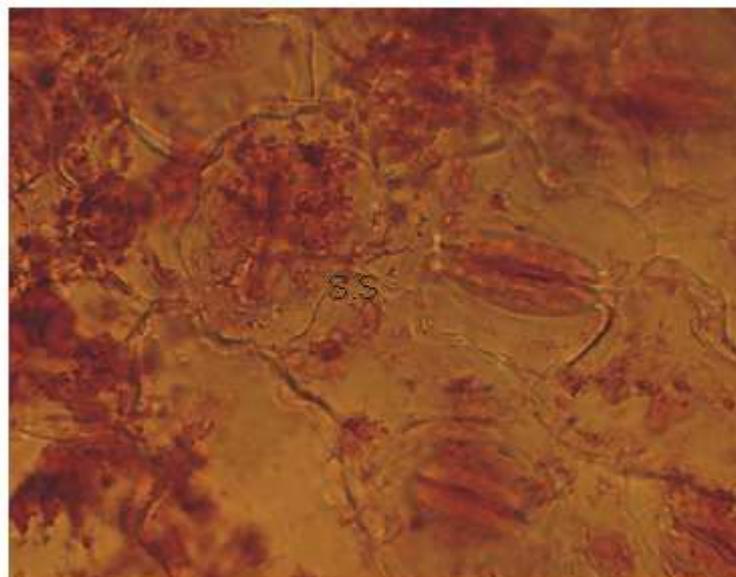


Plate 1C, S.S: Staurocytic Stomata of *Acalypha hispida* (lower surface)x400

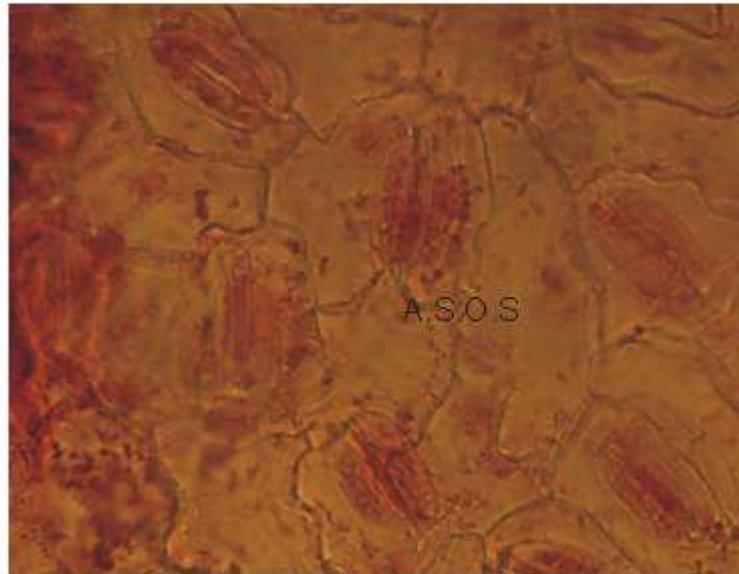


Plate 1D, A.S.O.S: Anisocytic sharing one subsidiary cell of *Acalypha hispida* (lower surface)x400

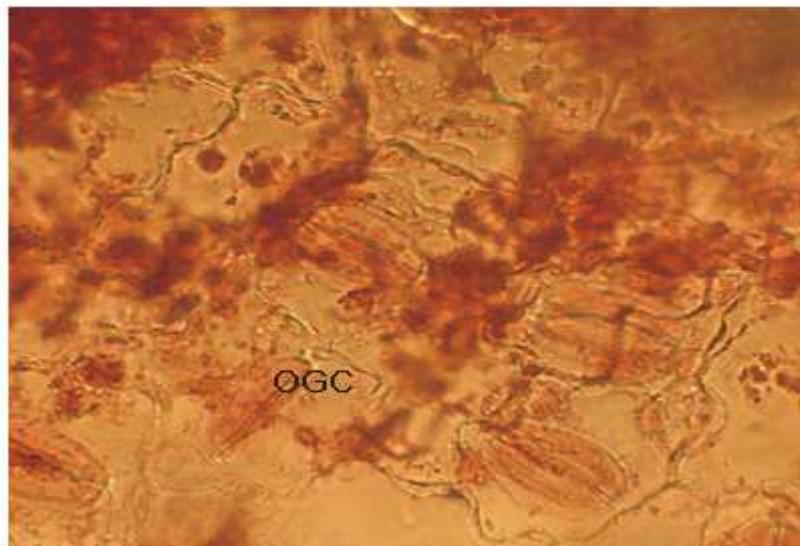


Plate 1E, O.G.C: One guard cell of *Acalypha hispida* (lower surface)x400

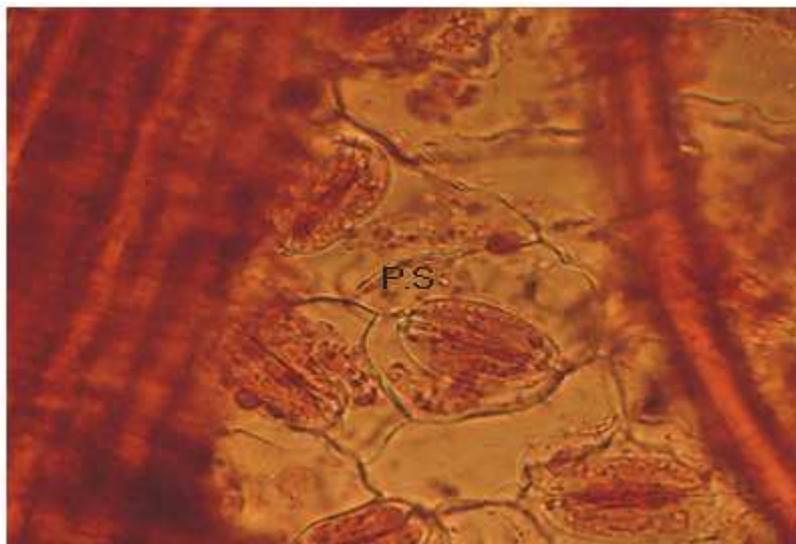


Plate 1F, P.S: Paracytic Stomata of *Acalypha hispida* (lower surface)x400

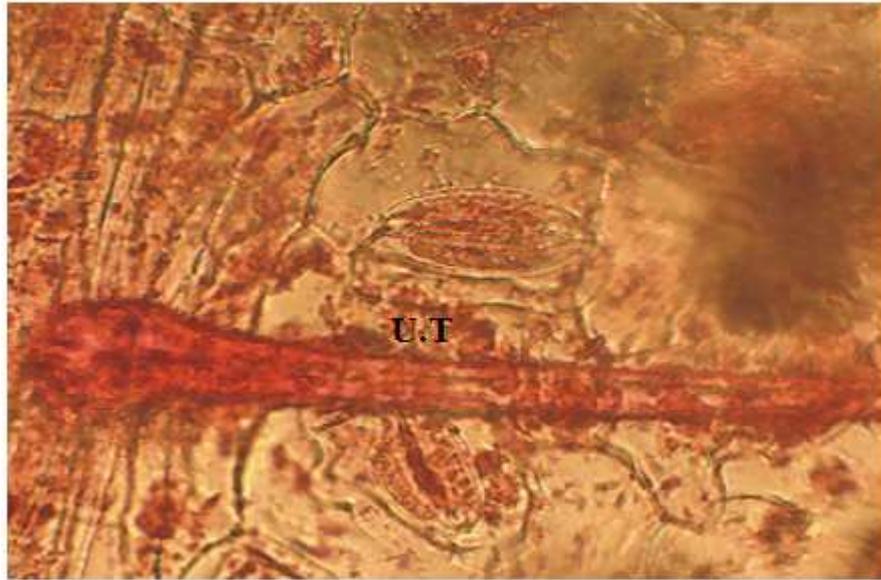


Plate 1G, U.T: Unicellular Trichome of *Acalypha hispida* (lower surface) x400

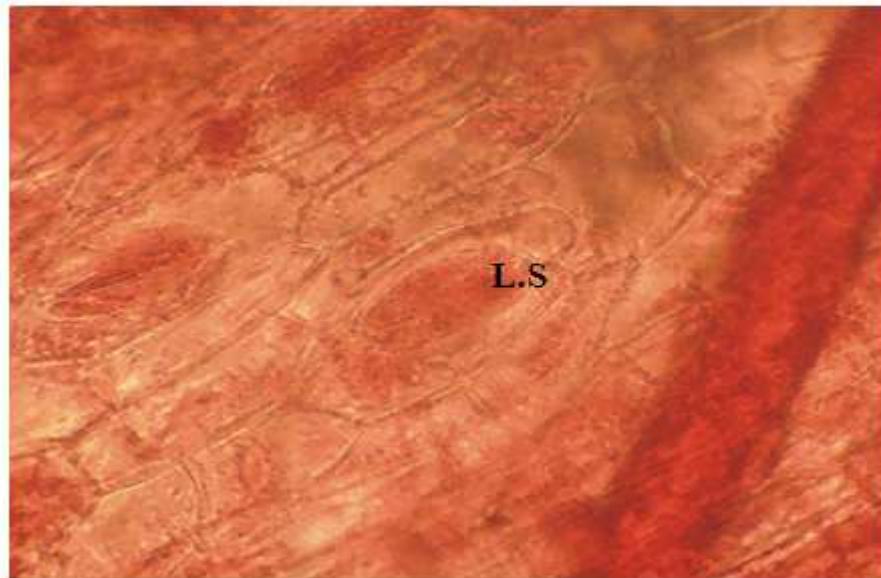


Plate 1H, L.S: Laterocytic Stomata of *Acalypha hispida* (upper surface)x400

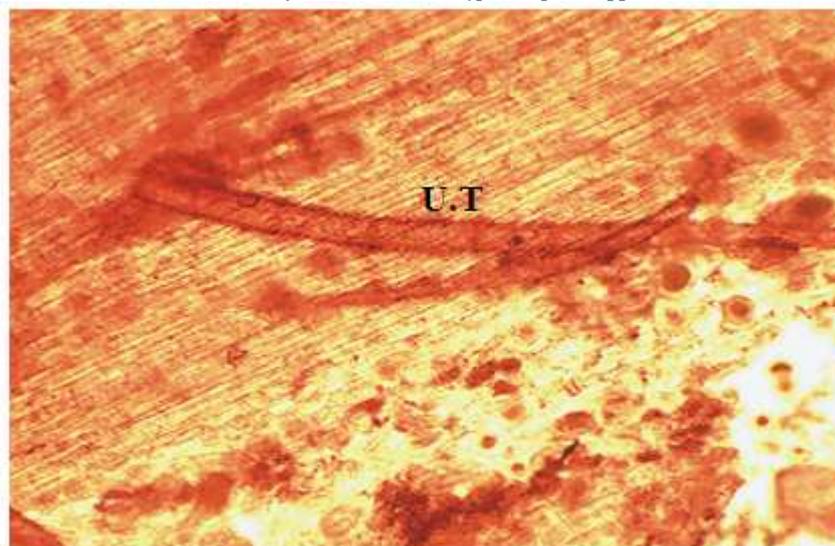


Plate 1I, U.T: Unicellular Trichome of *Acalypha hispida* (upper surface) x100

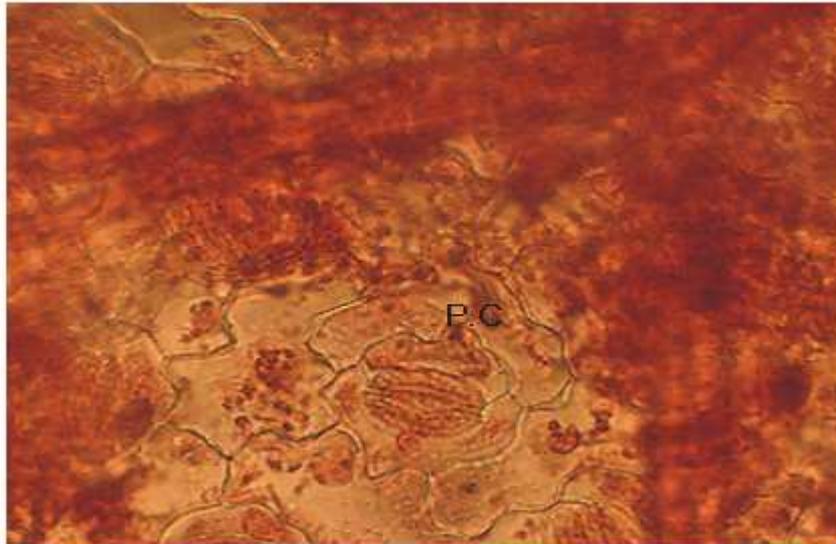


Plate 2A, P.C: Parallel contiguous stomata of *Acalypha goseffiana* (lower surface)x400

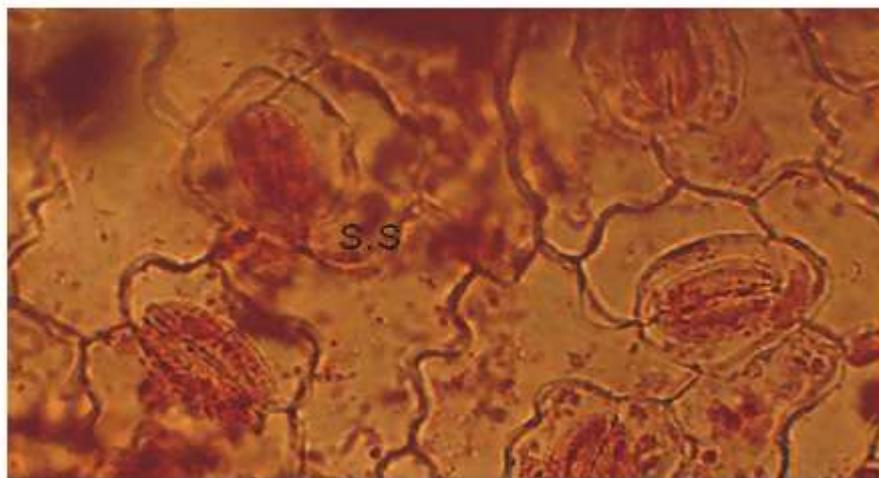


Plate 2B, S.S: Staurocytic Stomata of *Acalypha godseffiana* (lower surface)x400

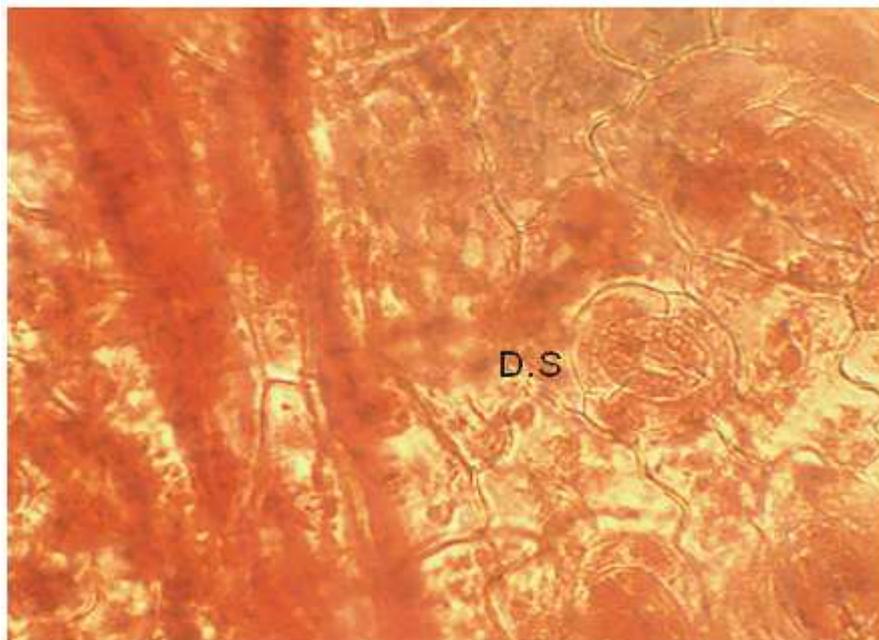


Plate 2C, D.S: Diacytic Stomata of *Acalypha godseffiana* (lower surface)x400

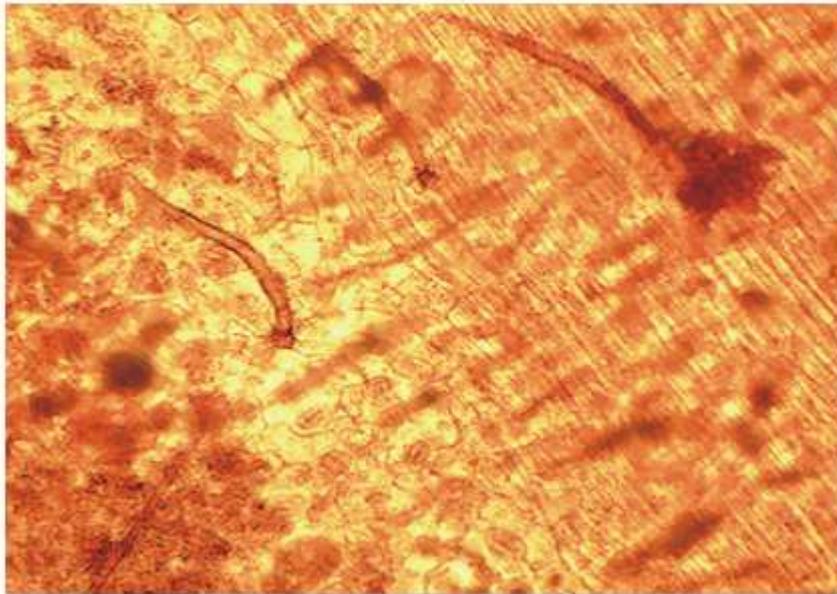


Plate 2D, U.T: Unicellular Trichomes of *Acalypha godseffiana* (lower surface) x100

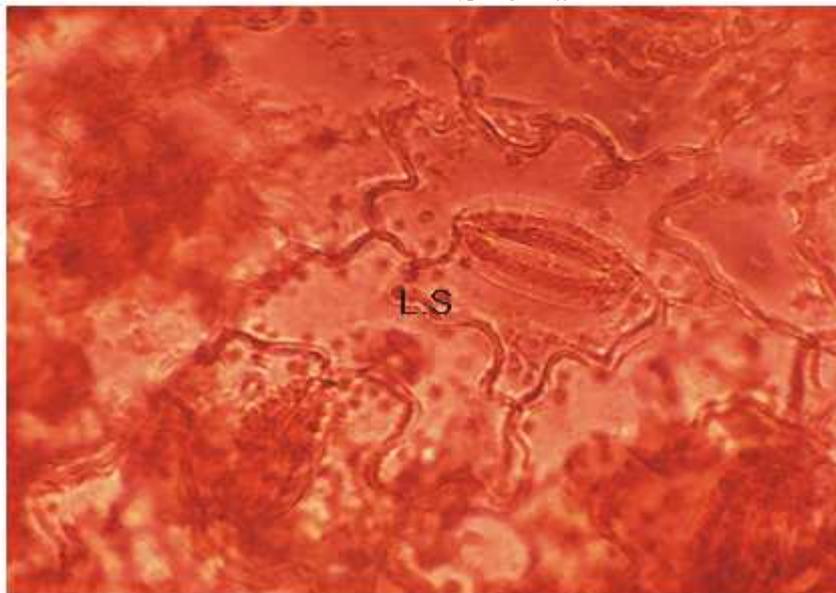


Plate 2E L.S: Laterocytic Stomata of *Acalypha godseffiana* (lower surface)x400

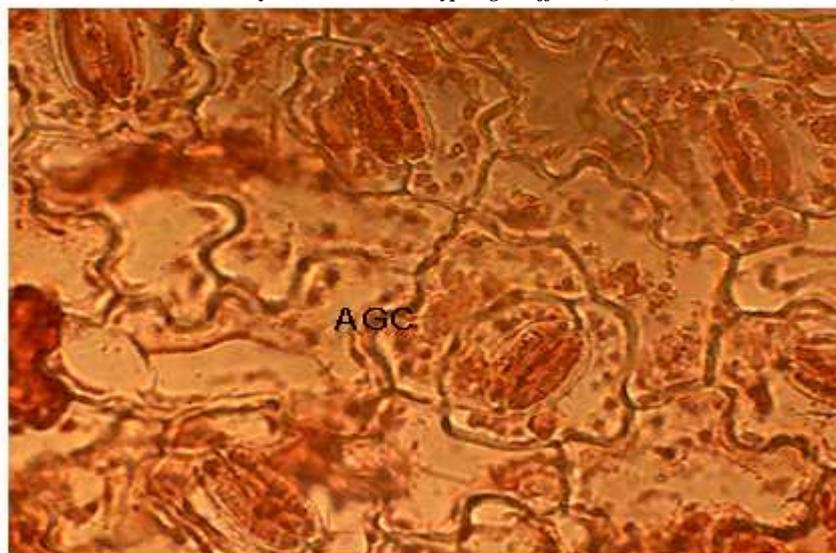


Plate 2G, A.G.C: Aborted guard cell of *Acalypha godseffiana* (lower surface) x400



Plate 2H, P.S. Paracytic Stomata of *Acalypha godseffiana* (lower surface) x400



Plate 2I, U.S.P: Unopen Stomata Pore of *Acalypha godseffiana* (lower surface)x400



Plate 2J, P.C: Parallel contiguous stomata of *Acalypha godseffiana* (upper surface)x400

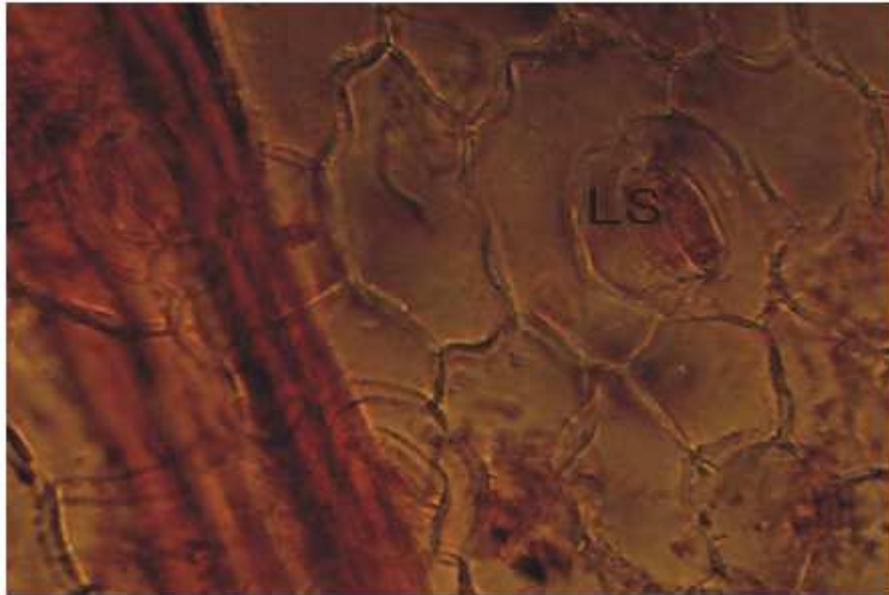


Plate 2K, L.S: Laterocytic Stomata of *Acalypha godseffiana* (upper surface)x400

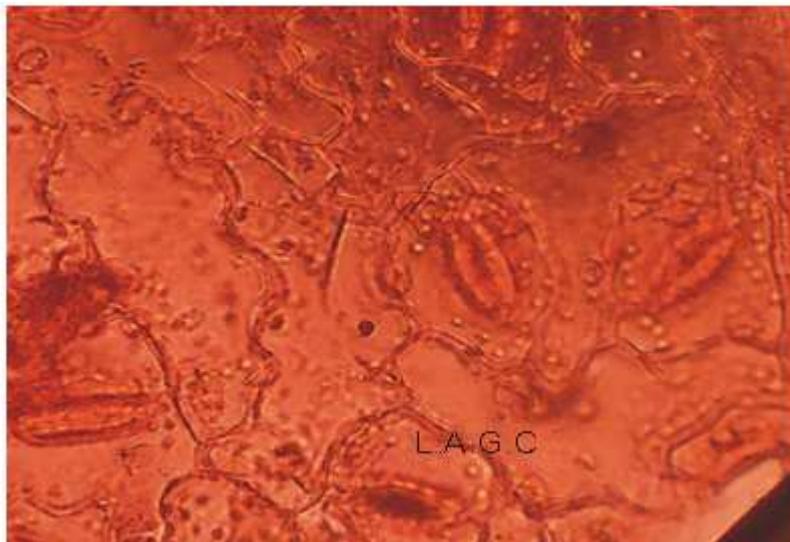


Plate 2L. L.A.G.C: Laterocytic and Aborted guard cell of *Acalypha godseffiana* (lower surface)x400



Plate 3A, U.T Uniseriate Trichome of *Acalypha wilkesiana* (upper surface) x400

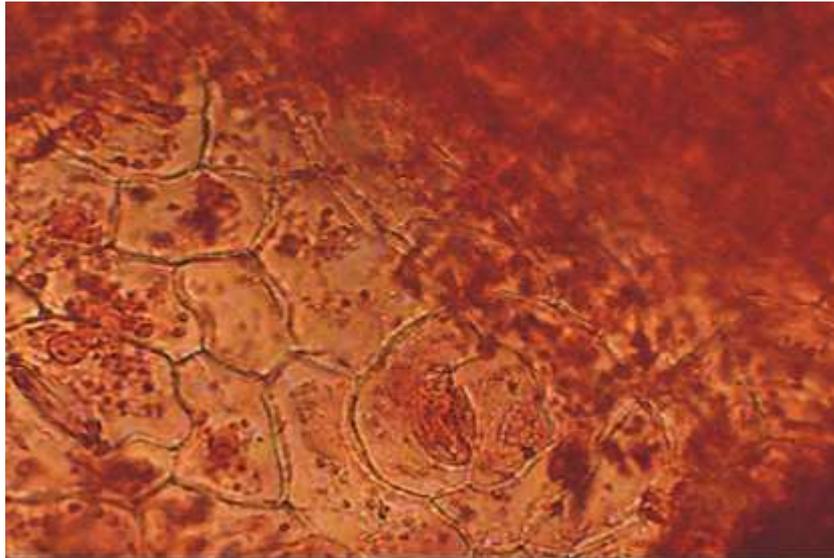


Plate 3B, O.G.C: One guard cell of *Laterocytic Acalypha wilkesiana* (lower surface)x400

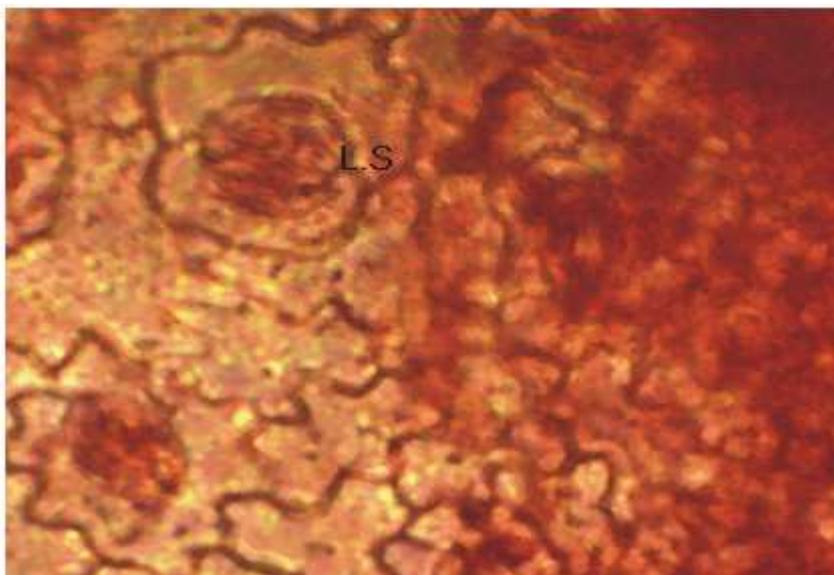


Plate 3C, L.S: Laterocytic Stomata of *Acalypha wilkesiana* (lower surface)x400

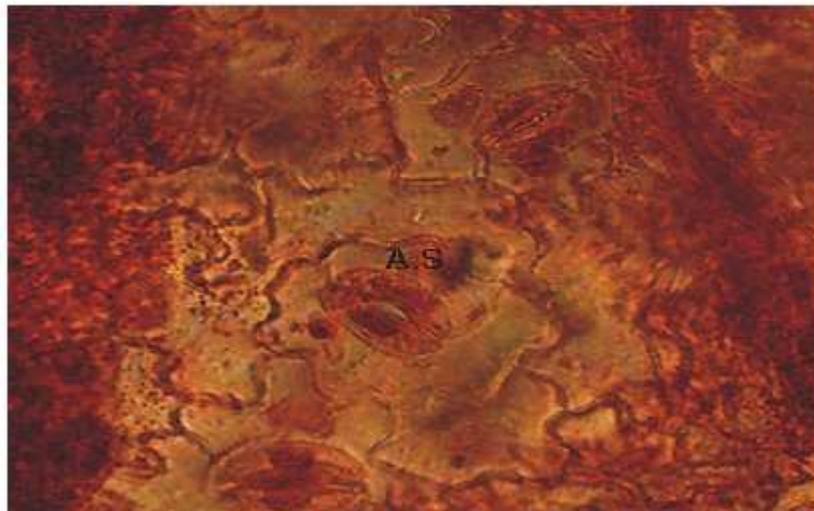


Plate 3D, A.S: Anisocytic stomata of *Acalypha wilkesiana* (lower surface x400

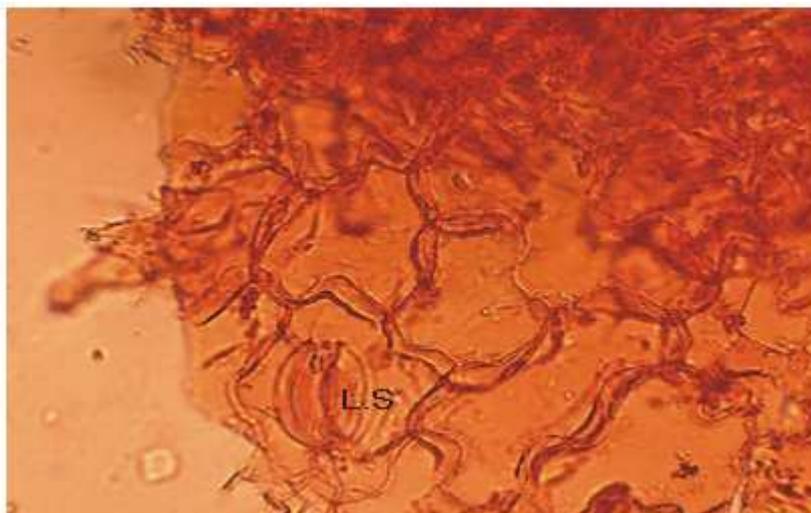


Plate 3E, L.S: Laterocytic Stomata of *Acalypha wilkesiana* (upper surface)x400

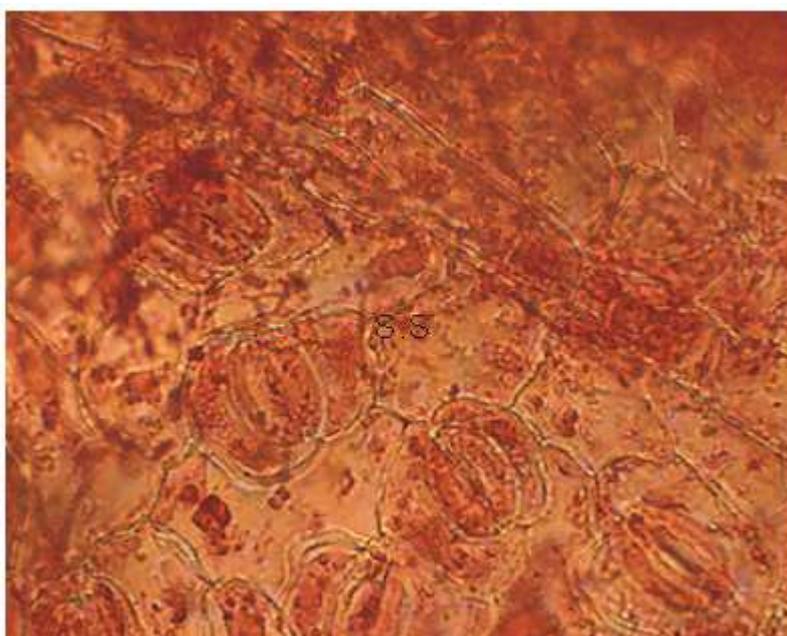


Plate 3F, S.S.: Staurocytic Stomata of *Acalypha wilkesiana* (lower surface) x400

DISCUSSION

The shapes of the epidermal cell vary from polygonal and irregular in all the three species while the Anticlinal cell walls were highly undulate in abaxial surface of *A wilkesiana* but undulate in *A hispida* and *A godseffiana* while the adaxial surfaces were straight to slightly undulating. The epidermal cell sizes vary significantly and can be fairly used for the separation of these species. The highest epidermal cell size was found in *A hispida* while the lowest was found in *A. wilkesiana*. The variations in stomatal index observed in this study can be reasonably employed in delimiting the *Acalypha* species (Table 1). Stace (1965), Metcalfe and Chalk (1979), Olatunji (1983), Adedeji and Jewoola (2008), Essienn and Akpabio (2009) have documented systematic relevance. The stomatal index and guard cell area have been found useful in diagnosis of some of the species, it ranges from 39.3x31.4 μm to 34.0x32.0 μm and the guard cell area ranges from 44.12 μm^2 to 39.5 μm^2 . *A godseffiana* can be distinguished by having parallel contiguous stomata on both surfaces.

Table 1: Result of Epidermal Features of *Acalypha* Species

Species	Stomatal size (μm)		Trichomes (lower and upper)	Epidermal cell size (μm)		Trichomes (μm)		Guard cell area (μm^2)		Stomatal Index (%)		Stomatal Distribution	Epidermal cell wall	
	Ab	Ad		Ab	Ad	Ab	Ad	Ab	Ad	Ab	Ad		Ab	Ad
<i>A. hispida</i>	15.5x9.1	16.3x 8.5	Present on both upper and lower surfaces	29.1x18.6	26.4x15.5	45.9x6.7	55.7x7.1	42.1	41.7	34.0x32.0	35.0x35.0	Hypo amphistomatic	Undulate	Straight to slightly undulating
<i>A. wilkesiana</i>	13.3x9.3	13.9x9.6	Present on both upper and lower surfaces	22.3x 18.3	20.9x16.9	51.5x7.4	63.6x7.0	39.5	41.9	37.3x33.6	39.9x36.2	Hypo amphistomatic	Highly-Undulate	Straight to slightly undulating
<i>A. godseffiana</i>	16.5x8.9	12.9x8.5	Present on lower and absent on upper surface	24.3x19.4	22.6x14.8	53.3x 7.3	-	44.1	40.4	39.3x31.4	35.9x28.0	Hypo amphistomatic	Undulate	Straight to slightly undulating
				<i>Ab</i>	=	<i>Abaxial</i>								
				<i>Ad</i>	=	<i>Adaxial</i>								
				<i>NG</i>	=	<i>Non-glandular</i>								
				<i>L</i>	=	<i>Length</i>								
				<i>B</i>	=	<i>Breadth</i>								

Some attempts have been made to use stomatal characters as an aid to classification. Aworinde (2009), Essiett (2004), Saheed and Illoh (2010) have reported the occurrence of more than one type of stomata on the same surface of an organ in a species. A combination of different types of stomata has also been observed here on the same surface of an organ in the different species investigated.

The morphology of stomata in *Acalypha* species were laterocytic, staurocytic, anisocytic, paracytic and diacytic which corroborate slightly with Aworinde *et al.* (2009). The occurrence of curved walls in these species agrees with the suggestion of Stace (1965) which says that curved walls is a mesomorphic character and that environmental conditions such as humidity play a significant role in determining cell wall thickness.

The relative abundance and variation of the trichomes is of taxonomic importance in the species. The presence of few long, short-pointed to curved apices unicellular non-glandular trichomes was found on vein cells and surrounding cells of both surface of *A. hispida* while few long, short pointed to curved uniseriate non-glandular trichome occurred on vein and surrounding cells of *A. wilkesiana* of both surfaces, unicellular with curved apices non glandular trichome were encountered only on the abaxial surface of *A. godseffiana* while both surfaces of *A. wilkesiana* and *A. hispida* had trichomes (Table 1). Although quantitative, the variations in trichomes length observed in this study can be employed in delimiting the species. Metcalf and Chalk (1979) hold that trichomes frequency is environmentally controlled. Many researches have found the presence or absence and types of trichomes on the epidermal surfaces as classificatory tool (Essiett, 2004; Adedeji and Jewoola, 2008).

Another interesting characters is the presence of abnormal stomatal cells with aborted guard cells, one guard cell, unopen stomatal pore and parallel contiguous stomata. Anomalous stomata found in *A. hispida* were aborted guard cell and one guard cell and two stomata sharing one subsidiary cells on abaxial surface, while those in *A. wilkesiana* are one guard cell on abaxial surface and those in *A. godseffiana* were one guard cell, aborted guard cell, and unopen stomatal pore on abaxial surface while parallel contiguous was found on both surfaces. The importance of abnormalities in leaves have been the result of environmental factors confirmed by Carr and Carr (1990).

CONCLUSION

The findings in the study of *Acalypha* species have indicated that some attempts can be made to use stomatal characters as an aid in classification. The presence of various stomata is of taxonomic interest in this study because it can be used to distinguish *Acalypha* species which is hypoamphistomatic.

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