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Adaptive Leaf-Architecture of the Epidermal Anatomical Characters of Four *Euphorbia* Species in Keffi, Nasarawa State.

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Abstract
Adaptive leaf-architecture of the epidermal anatomical characters was carried out on four species of Euphorbia found in Keffi, Nasarawa State. To assess the adaptive foliar-architecture of the epidermal anatomical characters to serve as a marker for the study of any characteristic changes, features understanding and coping mechanisms of the euphorbia species in the area. The studied plant samples were collected, identified, preserved and authenticated with a specimen number at National Institute for Pharmaceutical Research and Development (NIPRD). The anatomical characters studied shows various characters with respect to the presence of Anisocytic, Anomocytic and Paracytic stomata type in the species, stomata dimension of stomata mean Length (µm) ranged from 15.30to 25.70. While Stomata mean width (µm)ranged from9.52to16.32and Guard cell mean Length (at widest point) (µm) ranges from 4.42to 62.12. Presence of multicellular uniseriate trichomes in some species was demonstrated, while Trichome Mean Length (µm) ranged from 248.28to 862.24.polygonal , undulate and irregular to straight or slightly cell shape were present, while Epidermal mean Length (µm) ranges from31.62to 50.88,Epidermal mean width (µm) ranged from 17.34to 29.92. Feature in respect to lower density and size of stomates have been clarified as xeromorphic adaptations in respect to their low stamata index. The information from this study has indeed provided adaptive morphological and anatomical leaf architecture of the four (4) Euphorbia speciese specially for the less studied species from Keffi, Nasarawa State.

Keywords: Leaf-architecture, *Euphorbia* species, Keffi.

INTRODUCTION
In order to survive in different environments, the morphology and anatomical traits of widely distributed plant species often vary considerably. Leaves are exposed to aerial conditions more than any other plant organs, and the changes in their characters have been interpreted as adaptations to specific environments (Charles *et al*., 2003). Cell sizes, and stomatal density of leaves, among other features, are associated with environmental conditions (Magdalena *et al*., 1999). However, such environmental extreme conditions do characterize the morphological and anatomical characters of the plants thriving in those particular environments.

Depending on the requirement of water, Schimper (2010) has classified plants into three major groups. Mesophytes **(**plants adapted to a habitat with adequate Water), Xerophytes

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(plants adapted to a dry habitat) and Hydrophytes **(**plants adapted to a freshwater habitat). The genus *Euphorbia* is a genus of flowering plants belonging to the family Euphorbiaceae and consists of about 2008 species (Schimper, 2010). It is one of the largest and most diverse genera in the plant kingdom (Stebbins and Hoogland, 1976). The plants are annual or perennial herbs, woody shrubs or trees with a caustic poisonous milky sap (latex). Flowers in Euphorbiaceae are often very small in size. In the genus *Euphorbia*, the flowers are reduced even more and are aggregated into an inflorescence or cluster of flowers known as a cyathium. This feature is present in every species of the genus but nowhere else in the plant kingdom. Fruits of *Euphorbia* are capsules that typically split open when ripe. There are potentially three seeds per capsule, and there is a wide variety of size, shape and surface features of the seeds and capsules (Davis *et al.,* 2007).

Euphorbia continifolia is a broadleaf red shrub native to Mexico and South America. Treated as a shrub, it reaches 10 to 15ft (3.0 to 4.6 m) but can be grown as a tree reaching 30 ft (9.1 m). *Euphorbia hirta* (sometimes called asthma-plant) is a pantropical weed, possibly native to india.it is a hairy herb that grows in open grasslands, roadsides and pathways. It is used in traditional herbal medicine. *Euphorbia milii*, the crown of thorns, Christ plant, or Christ thorn, called Corona de Cristo in Latin America, is a species of flowering plant in the spurge family Euphorbiaciae, native to Madagascar. *Euphorbia thymifolia* is a prostrate annual plant producing stems up to 25cm long. The stems usually produce numerous adventitious roots (Aworinde*et al*., 2009).

In view of the above considerations, it was thought worthwhile to examine the adaptive features of the leaves epidermal anatomical characteristics responsible for the coping mechanisms of euphorbia species in Keffi, Nasarawa State. Also, to clarify features in respect to the density and size of stomates in regard to implication as species adaptations and to observe and study any form of changes on the morphological and epidermal anatomical characters of the euphorbia species due to the environmental effect.

MATERIALS AND METHODS

Plant Collection and Voucher Specimen Number

The plant samples were collected from Keffi Local Government Area of Nasarawa State. Specifically, the plant samples of the four *Euphorbia* L. species were collected from Nasarawa State University campus Keffi. *Euphorbia cotinifolia* was collected around School of Post Graduate, *Euphorbia hirta* around staff quarters, *Euphorbia thymifolia* was collected around mande boys hostel, while *Euphorbia milii* was collected around faculty of administration on the same campus. They were pressed and stored in the herbarium of National Institute for Pharmaceutical Research and Development (NIPRD) and were authenticated with a specimen number.

Preparation of Samples for Foliar Epidermal Studies

The Wilkinson's (1979) standard leaf clearing procedure was used with slight modification. The work bench was cleansed using cotton wool and ethanol before the specimens (matured leaves) were placed on it. About 1mm to 1cm squared leaf fragment were obtained from the standard median portion of the leaf and macerated in concentrated trioxonitrate v acid in petri-dish for a period of about 24h. The appearance of bubbles on the surface of the leaf fragments indicated their suitability for separation. They were transferred in to water in petri-dish with a pair of forceps. Both epidermises were carefully separated by teasing them apart and pulling each epidermis back on itself. The epidermises were cleaned with the

carmel hair brush. These were rinse in distilled water and later transferred in to 50% ethanol for about two minute to harden. They were then mounted on glass slide and few drop of glycerol is added after tissue paper was used to drain off the water from the epidermises when it has been transferred to the slide gently. The slides were labeled appropriately and examined under the light microscope while photograph of the micro morphological features were taken using microscope with camera attached at a magnification of x400.

For statistical analysis, five (5) epidermal cells and five (5) stomata were chosen at random from each species and measured using a micrometer eyepiece with calibration factor of 3.4 µm. For each quantitative character, the range, mean, standard deviation and standard error were determined for each species.

The stomata Index was determined according to Metcalfe and Chalk (1979) using the formula

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

Where:
S = Number of stomata per unit area
E = number of epidermal cells in the same area.

The foliar epidermal characters studied include:

1. **Epidermal Cell Complex**
   Epidermal cell shape
   Anticlinal wall pattern
   Epidermal cell size (length and width)
   Epidermal cell frequency

2. **Stomata Complex**
   Stomata type
   Stomata size (length and width)
   Stomata index
   Guard cell (width at the widest point)

3. **Trichome morphology**
   Trichome type
   Trichome size (length and width)
   Trichome distribution

RESULTS AND DISCUSSION

Foliar Epidermal Anatomical Characters

The parameters of key epidermal features which include stomata complex, epidermal cell complex and trichome morphology were critically studied and evaluated as described below.

List of Plates Showing Leaf Epidermal Characters

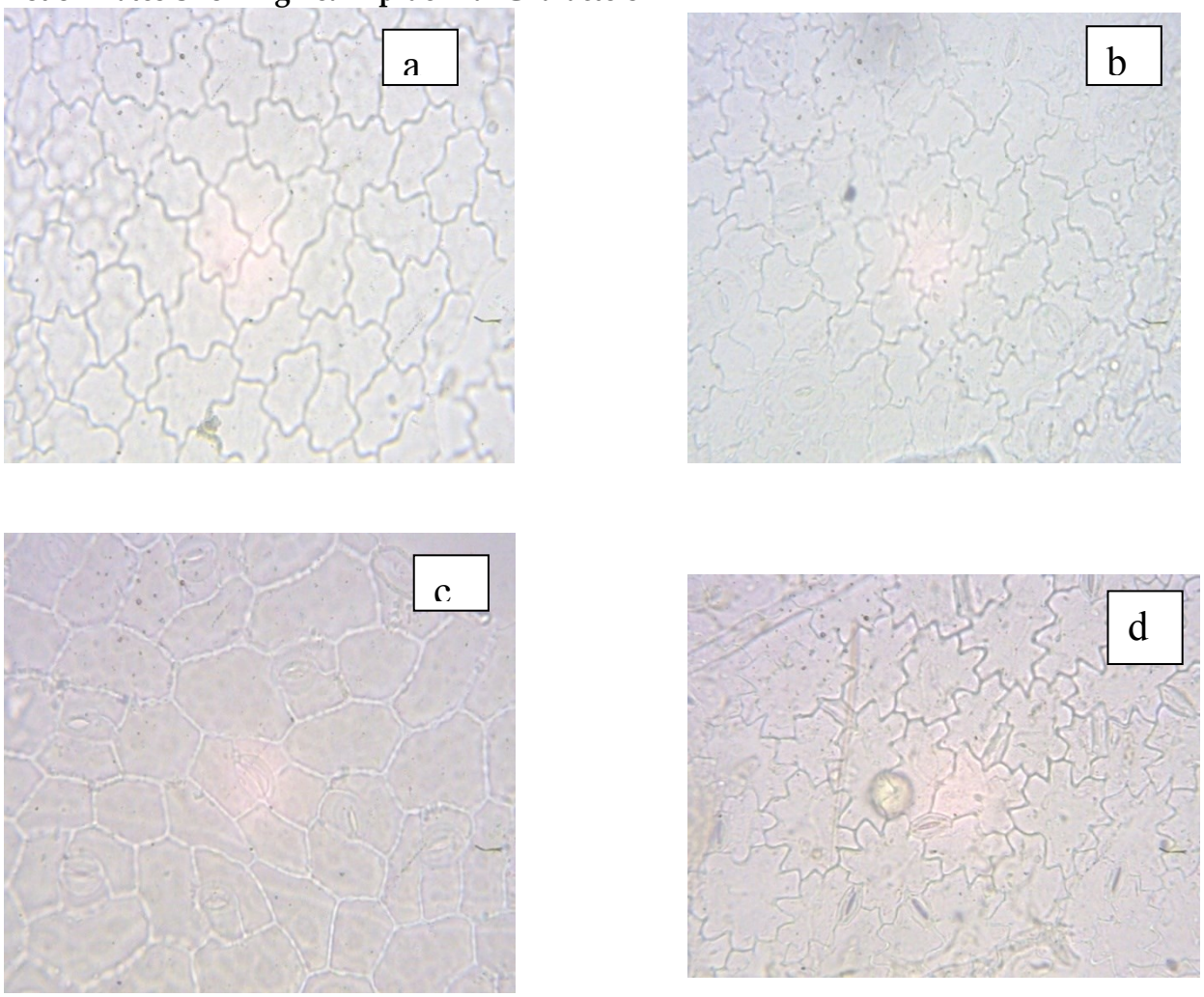

PLATE 1: Plates showing Leaves Epidermal Characters (X400)

Plate 1a: Undulate epidermal cells on the upper surface of *Euphorbia cotinifolia*.

Plate 1b: Anomocytic Stomata, Anisocytic Stomata, and Irregular Epidermal cells observed on the lower surface of *Euphorbia cotinifolia*.

Plate 1c: Anisocytic Stomata, and Polyhedral Epidermal cells observed on the upper surface of *Euphorbia hirta*. X400

Plate 1d: Anomocytic, Anisocytic types of stomata, and Wavy Epidermal cells observed on the lower surface of *Euphorbia hirta*.

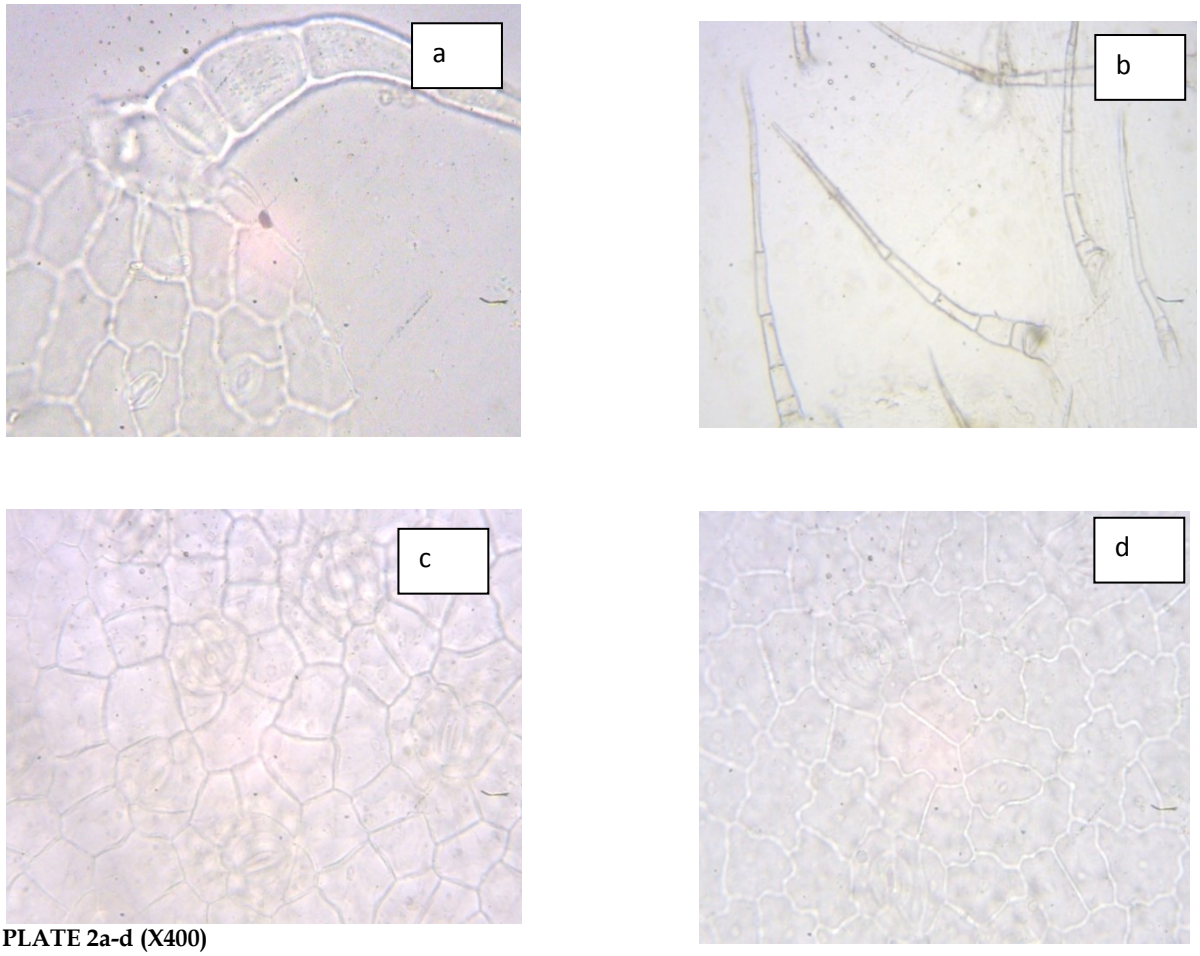

PLATE 2a-d (X400)

Plate 2a: Multicellular Uniserriate Trichome observed on the upper surface of *Euphorbia hirta.*
Plate 2b: Multicellular Uniserriate Trichome observed on the lower surface of *Euphorbia hirta.*
Plate2 c: Paracytic Stomata and Polygonal Epidermal cells observed on the upper surface of *Euphorbia milii*.
Plate 2 d: Paracytic Stomata and Undulate Epidermal cells observed on the lower surface of *Euphorbia milii*.

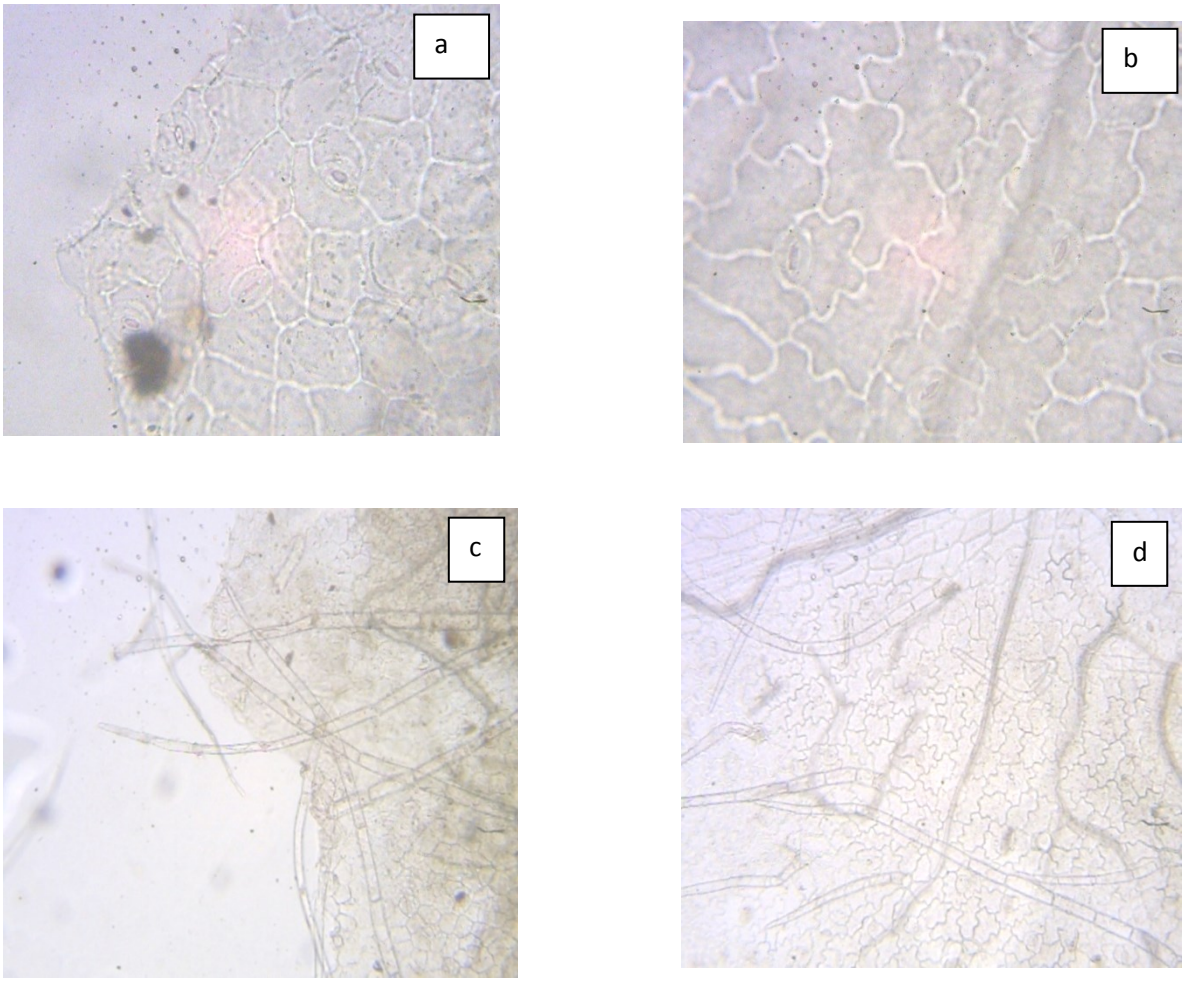

PLATE 3a-d (X400)
Plate 3a: Anisocytic Stomata and Polygonal Epidermal cells observed on the upper surface of *Euphorbia thymifolia.*
Plate3b: Anisocytic Stomata and Undulate Epidermal cells observed on the lower surface of *Euphorbia thymifolia*.
Plate3c: Multicellular Uniserriate Trichome observed on the upper surface of *Euphorbia thymifolia*.
Plate3d: Multicellular Uniserriate Trichome observed on the lower surface of *Euphorbia thymifolia.*

Table 1: Qualitative foliar micro morphological characters of the studied *Euphorbia species*

Species	Epidermal cell shape		Anticlinal wall pattern		Stomata type		Trichome type	
	Ad	Ab	Ad	Ab	Ad	Ab	Ad	Ab
E. cotinifolia	Undulate	Irregular	Wavy	Wavy	-	Anisocytic/ Anomocytic	-	-
E. hirta	Polyhedral	Undulate	Straight/ slightly curved	Wavy	Anisocytic	Anisocytic/ Anomocytic	Multicellular uniserriate	Multicellular Uniserriate
E. milli	Polygonal	Irregular/ Undulate	Straight/ slightly curved	Straigh t/sligh tly curved	Paracytic	Paracytic	-	-
E. thymifolia	Polygonal	Undulate	Straight/ slightly curved	Wavy	Anisocytic	Anisocytic	Multicellular uniserriate	Multicellular Uniserriate

Ad: Adaxial Ab: Abaxial

Table 4: Quantitative anatomical dimension of the studied *Euphorbia species*

Species		Stomata dimension Length (µm)	width (µm)	Epidermal dimension Length (µm)	width (µm)	Trichome dimension Length (µm)	Guard cell dimension Length (at widest point) (µm)
E. cotinifolia	*Ad*	-	-	31.96±1.73	20.06±1.13	-	-
	Ab	22.50±0.63	16.32±0.42	31.62±1.27	17.34±1.36	-	6.12±0.42
E. hirta	*Ad*	18.54±0.67	11.56±0.83	51.68±1.98	28.56±1.73	248.28±24.54	4.42±0.42
	Ab	15.30±0.76	9.52±0.42	42.16±1.73	26.86±1.13	515.10±53.86	4.42±0.42
E. milli	*Ad*	21.08±1.27	11.56±0.83	38.76±2.04	22.44±1.73	-	5.10±0.76
	Ab	25.70±0.66	16.32±0.42	40.12±1.27	24.14±1.13	-	8.16±0.64
E. thymifolia	*Ad*	18.36±0.83	12.24±0.64	48.96±1.36	26.52±1.27	862.24±138.75	52.78±0.4
	Ab	22.44±0.64	12.92±0.42	50.88±1.59	29.92±2.49	368.56±75.62	62.12±0.4

Ad: Adaxial Ab: Abaxial

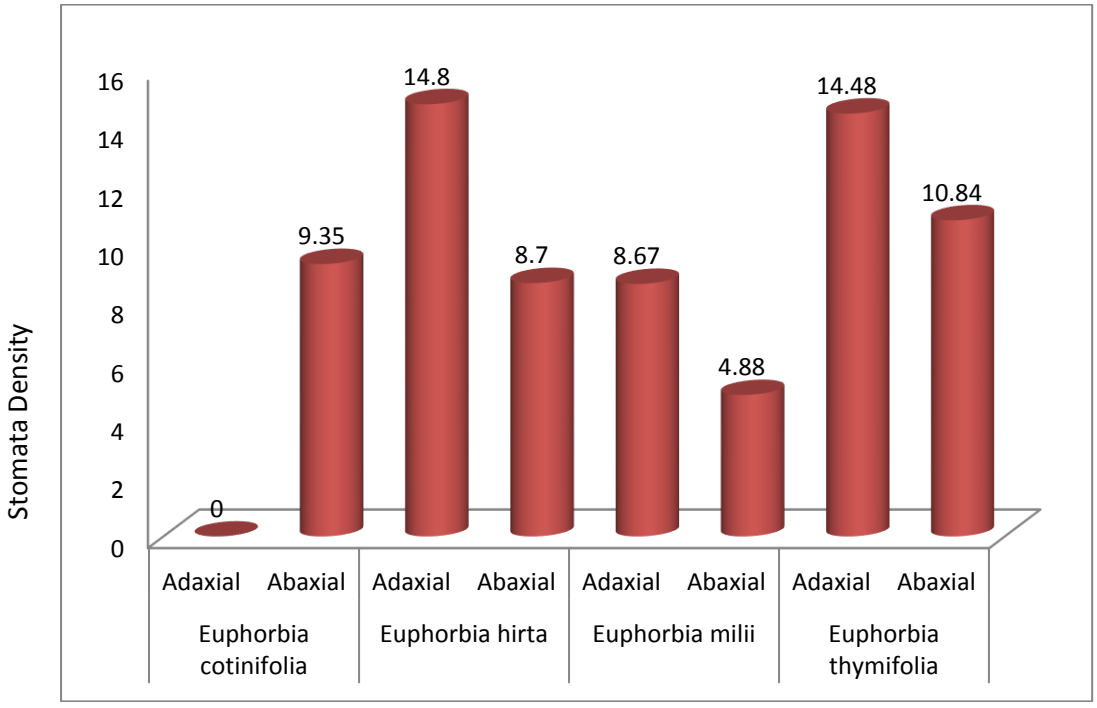

Figure 2: Stomatal density of *Euphorbia* species

This study shows a number of important macro and micro morphological characters on the leaf surfaces of four studied *Euphorbia* species. Although, new characteristics were observed

that were not previously recorded, generally there is a disagreement and an agreement in the results obtained from this present study and previous studies.

Stomata are tiny microscopic pores or apertures found on the epidermis of leaves that helps in regulating loss of water via transpiration, and carbon IV oxide uptake during photosynthesis (Bussis *et al.*, 2006). The pores are bordered by specialized parenchyma cells known as guard cells that are responsible for regulating the size of the opening of the stomata. (Nwachukwu and Mbagwu, 2006). Diversity of stomata was reported by Akuodor*et al.* (2013) in the *Euphorbia* species studied. This is in agreement with Raju and Rao (2008), Aworinde *et al.* (2009), Saheed and Illoh (2010) and Akuodor*et al.* (2013) who also observed the occurrence of more than one stomata type on a leaf surface.

Cuticular examination revealed the presence of stomata on both the abaxial and adaxial surfaces of the species studied with higher frequency on the adaxial surfaces except in *Euphorbia cotinifolia* with no stomata on the adaxial surface. This in agreement with the findings of Silva (2006), Akuodor*et al.* (2013) who observed stomatal complexes more on the adaxial surfaces in the species studied. The stomata measurement reveals that *Euphorbia hirta* has the smallest stomata length (17.00µm – 20.40µm and 13.60µm – 17.00µm on the adaxial and abaxial surfaces respectively) while *Euphorbia milii* has the highest stomata length (17.00µm – 23.80µm and 23.80µm – 27.20µm on the adaxial and abaxial surfaces respectively).

The density and size of stomates have been implicated as xeromorphic adaptations. Raju and Rao (2008) stated that stomatal densities are decreased in xerophytes plants. With fewer stomates per unit surface area there would be less transpiration, a favorable adaptation in a xeric environment. Bussis *et al.*(2006) stated that there is no correlation between the density of stomates and the xeric habitat. Bussis *et al.*(2006) stated that xerophytes plants may actually have an increased stomatal density. Raju and Rao (2008) stated that stomatal density is decreased while Akindele and Adeyemi (2007) state that densities are increased in xerophytic plants. Strobel and Sundberg (2014) carried out an experiment and suggested that succulent and non succulent xerophytes evolved different stomatal strategies to deal with water stress.

However, in this present study, the stomata index found on the adaxial and abaxial surface of the *Euphorbia* species *respectively are; Euphorbia milii* with an index of 8.67% and 4.88%, *Euphorbia hirta* 14.80% and 8.70%, *Euphorbia thymifolia*, 14.48% and10.84% while E*uphorbia cotinifolia* was found with stomata index of 9.35% on the abaxial surface only. Therefore, this study is in agreement with the surgestion and findings of Ting (1982), Maximov (1929),Martins and Zieri (2003) and Belhadj *et al.*, (2007) who stated decrease number of stomatal densities in xerophytes and also agrees with the findings of Strobel and Sundberg (1984) who suggested that succulent and non succulent xerophytes evolved different stomatal strategies to deal with water stress and contradict the work of Akindele and Adeyemi (2007) who stated that densities are increased in xerophytic plants and also disagrees with the work of Volkens (1884) who stated that there is no correlation between the density of stomates and the xeric habitat.

Metcalfe and Chalk (1979) hold that the trichome frequency and size are environmentally controlled, The presence or absence, type, size as well as the distribution of trichomes can be used in environmental control. This study show the presence of multicellular uniseriate trichomes on both surfaces of *Euphorbia hirta* and *Euphorbia thymifolia.* This is in agreement

with Silva (2006) who reported multicellular uniseriate trichomes in *Euphorbia hirta* and *Euphorbia thymifolia*. Furthermore, the sizes of trichomes on the *Euphorbia* species vary with *Euphorbia thymifolia* having the longest trichome on the adaxial surface and *Euphorbia hirta* having the shortest trichome on the adaxial surface.

Stace (1989) stated that curved wallhas a mesomorphic characteristics and that environmental conditions such as humidity play a significant role in determining the pattern of anticlinal cell wall. In this present study, the anticlinal cells are wavy on the abaxial surface for *Euphorbia hirta* contradicting the findings of Akuodor*et al.* (2013) who reported anticlinal walls with highly undulated cells on the abaxial surface of the species. However, this might be due to environmental effect explaining the difference encountered in the anticlinal cells of *Euphorbia hirta* stated.

The epidermal cell shape and anticlinal wall pattern varied within the studied species. *Euphorbia cotinifolia* L has undulate and irregular cell shape on the adaxial and abaxial surfaces respectively with wavy anticlinal cells on both surfaces. Also, *Euphorbia hirta* L and *Euphorbia thymifolia* L, both have polygonal and undulate cell shapes on their adaxial and abaxial surfaces respectively, follow by straight or slightly curved cell walls on their adaxial surfaces with wavy cell walls on their abaxial surfaces were observed. Similarly, *Euphorbia milii* have different epidermal cell shape and anticlinal wall pattern with polygonal and irregular cell shape on adaxial and abaxial surfaces respectively follow by straight to slightly curved anticlinal walls on both surfaces were observed.

The occurrence of irregular, undulate, polygonal and polyhedral epidermal cell shape is in agreement with the works of Silva (2006), Ahmad *et al.* (2010) and Akuodor*et al,* (2013) who reported the presence of irregular, polygonal or variously elongated epidermal cells in the *Euphorbia* species studied.

CONCLUSION

The information from this study has indeed provided adaptive morphological and anatomical leaf architecture of the four (4) *Euphorbia* species which include *Euphorbia cotinifolia, Euphorbia hirta, Euphorbia milii,* and *Euphorbia thymifolia* especially for the less studied species from Keffi, Nasarawa State.

The diagnostic characters used for species architectural description in leaf morphological study were, leaf type, leaf shape, leaf arrangement, leaf base, leaf apex and leaf size. While that of anatomical study were stomata type, stomata size, stomata index, epidermal shape and size, presence or absence and type of trichome.

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