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Foliar anatomical variation of *Triplochiton scleroxylon* k. schumin selected forest cover types in Akure Forest Reserve, Ondo State, Nigeria

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Abstract

This study assessed the foliar anatomical characteristics exhibited by Triplochiton scleroxylon from three selected forest cover types in the Akure forest reserve. The study was carried out in Queen's Plot, Enrichment Plantation, and Disturbed forest areas in Akure Forest Reserve, Ondo State. Five Triplochiton scleroxylon trees at not less than 50 meters apart were selected randomly, and three well-expanded leaves covering up, down, and the middle were collected, making 45 leaves in total, from the three selected forest cover types, they were all preserved in formalin acetic-alcohol (FAA) prior to the study, and their GPS coordinates were recorded. The result from the three selected land use types gives a regular polygonal reticulate venation pattern with a polygonal areolation shape in all three land use types. On leaf epidermal study from the adaxial surface, all the selected land use types possess similar qualitative characteristics; epidermal papillae were present only in accessions from Queen's plot, and stomata were absent from the three land use types, whereas on the abaxial surface, stomata type and structure are hypostomatic with paracytic stomata, possess polygonal epidermal cell shape, sparse crystal granules, and crescent guard cells for the three land use types, and the anticlinal wall is wavy in Queen's plot but sinuous in others. Also from the transverse section of leaf microanatomy, the palisade mesophyll layer present in disturbed forest areas was thicker than others, which is an adaptation to reduce water loss since the area is open, unlike Queen's plot, where the tree crown has covering and accession in enrichment plantation is the least. The petiole anatomy results show the presence of the uniseriate epidermis, angular collenchyma, and oval or polygonal parenchyma cells. The pith regions were all found to have specific characters as they occurred in all 45 accessions, which is considered taxon-specific. The presence of higher trichome density in all accessions may be advantageous to plants as it increases resistance against herbivory.

Keywords: *Triplochiton scleroxylon*, foliar anatomy, Queen's plot, enrichment plantation, disturbed forest area.

Introduction

Triplochiton scleroxylon (Family: Sterculiaceae), also known as "Obeche or Arere" (Yoruba, Nigeria), is an environmentally and economically important African tropical tree. Ayous (*Triplochiton scleroxylon* K. Schum), is an important timber species from the tropical West and central African forest, with approximately 38,000 m^3 traded annually[1]. It was reported that Obeche has a relatively acceptable broad leaf structure and unique stem traits including a self-pruned cylindrical bole of about 30m, a strong anchorage buttress of about 6m, and a trunk diameter of around $1.5m^2$. Leaves are palmately netted when they have several dominant veins branching out from the petiole and are 10–20cm long and broad. The lobes are broadly ovate, triangular or oblong, and rounded or obtusely acuminate at the apex; they are glabrous, and the stalk is 3–10cm long. The leaves of coppice and sapling branches are frequently bigger and more deeply lobed than the leaves of the crown. Obeche wood is commonly used for sculpting, hive construction, energy production, and carpentry[3]. Report from research reported that Ayous wood are used for varieties of purpose, from construction to ornamental objects to more specialized usage like sauna paneling and table tennis bats[2,4,5]. The bark is employed in traditional medicine to treat oedema, chickenpox, and as an analgesic, as well as to cover walls and roofs with boxes[6]. Strong anthropogenic pressure stemming from this ethno-botanical significance threatens the extinction of this species.

The invention of the microscope gave biologists a new instrument for studying the inside structure of organs and tissues, which led to the first application of anatomical traits in taxonomy[7]. It was found that morphological and anatomical characteristics both have value. Numerous characteristics that have been employed for taxonomic purposes are present in all portions of a plant. Scientists who are asked to identify small samples or scraps of plant material for specific purposes, such as pharmacognosists in the determination of a drug's source or forensic experts who may be able to provide clues to a crime investigation, among others, will find the aspect of plant anatomy to be of great importance[7]. Findings shows that, leaf anatomical attributes serve as significant markers of leaf traits and change along environmental gradients[8,9]. This indicates that plants can adjust their leaf anatomical structure ratios to respond to environmental changes. Studies reported that leaf epidermis and mesophyll characteristics demonstrate substantial spatial trends at the species and community levels[10-12]. For instance, as temperature and precipitation increased, the palisade mesophyll to leaf thickness ratio increased but the spongy mesophyll to leaf thickness ratio dropped[13]. The thickness of the epidermis and leaves in the Northern Hemisphere displayed latitudinal patterns of initially decreasing and then rising[14].

Materials and Methods

The three selected (forest cover type) study area was Queen's Plot, in Akure Forest Reserve is a strict nature reserve located between longitude 5˚1'48"E to 5˚3'42"E and latitude 7˚13'47"N to 7˚17'45.6"N in Ondo State. Enrichment plantation in Akure Forest Reserve of the is around 66km^2 (25 square miles), with some of it classified as a formal nature reserve since 1954 and other parts set aside for artificial regrowth in the forest's deteriorated areas. It is located between N07º.2645ˡ, and E0050.03675ˡ. A significant area of the forest reserve was known to have undergone a number of human operations before enrichment plants were started, including timber logging and exploitation by illicit fellers. However, a portion of the tight nature reserve close to that region had been set aside for forest regeneration by 2004. The goal of the forestry-assisted initiative known as the regeneration technique of enrichment plantation, which was developed in 2004 under the supervision of the Federal Government of Nigeria, was to restore the forest by planting regenerative trees. Disturbed forest area in Akure Forest Reserve are where a lot of anthropogenic activities are carried out (farming, felling, and harvesting of trees; fetching wood for fuel wood; collection of NTFP (non-timber forest products) such as tree bark, fruit, and leaves for medicinal purposes; young tree leaves for soup; hunting for wild animals for meat), which makes the forest area less populated by trees.

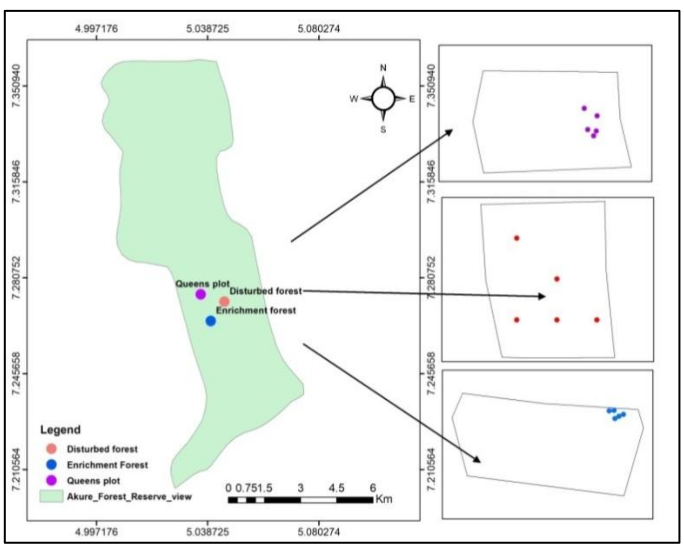

Figure-1: Sampled points in queens plot, enrichment plantation and disturbed forest area in Akure Forest Reserve for foliar anatomical study of *Triplochiton scleroxylon*.

Three (3) leaves each were collected from five (5) randomly selected Obeche trees from Queens Plot (SNR), Enrichment Plantation, and Disturbed Forest Land Area in Akure Forest Reserve, and their GPS coordinates were taken. Three well-expanded leaves covering up, down, and middle from each of the five trees at not less than 50 meters apart in each of the three locations were collected for the study, making 45 leaves in total. The leaves and their petioles were all preserved in formalin acetic acid (FAA) prior to study.

Table-1: List of coordinate points for Obeche trees sampled in Queens Plot (SNR) for foliar anatomical study.

S/N	Coordinate Points	
	Latitude	Longitude
1	N07º.27466	E005º.03618
2	N07º.27475	E005º.03609
3	N07.27473	E005º.03622
4	N07º.27495	E005º.03623
5	N07º.27506	E005º.03604

Table-2: List of coordinate points for Obeche trees sampled in Enrichment plantation for Foliar Anatomical Study.

S/N	Coordinate Points	
	Latitude	Longitude
1	N07º.26490	E005º.03989
2	N07º.26486	E005º.03979
3	N07º.26481	E005º.03970
4	N07º.26499	E005º.03968
5	N07º.26498	E005º.03957

Table-3: List of coordinate points for Obeche trees sampled in Disturbed forest area for Foliar Anatomical Study.

S/N	Coordinate Points	
	Latitude	Longitude
1	N07º.26511	E005º.03969
2	N07º.26515	E005º.03972
3	N07º.26524	E005º.03968
4	N07º.26690	E005º.03935
5	N07º.26860	E005º.03917

Reagents used for the study are: ethanol, sodium hydroxide, sodium hypochlorite (domestic bleach), safranin O, water, glycerol, conc. Nitric acid, toluidine blue, and alcian blue.

The instruments used for the study are: GPS device, microscope slide, cover slip, Accu-scope Trinocular Microscope (Accu-scope 33001 LED Trinocular Microscope with 4.2 MP CMOS Digital Camera), light microscope (Leica Galen III), Reichert Sliding Microtome (Reichert Austria Nr. 367 019) for sectioning, and ocular micrometers (linear and square).

Procedure: Standard median portions (midway between the tip and the base) were taken from each sample. The portions were put into Nitric Acid, in glass Petri-dishes, are kept in an oven at 60^0 C for 20minutes. Each sample was washed thoroughly in 4-5 changes of water. The abaxial and the adaxial epidermis were separated by means of fine forceps and dissecting needle. The epidermis were then stained in Safranin-O, and counter stained in Toluedene blue for five minutes, washed with 4-5 changes of water to remove excess stain and then mounted in 25% glycerol. The scrape method was utilized in getting the epidermal peels of some of the accessions[15]. The entire leaves were placed face down on a slide and flooded with a commercial bleaching agent (containing Sodium Hypochlorate). The material was then carefully scraped with a new razor blade until the epidermis was reached. The bleaching agent acted as lubricant and at the same time helped to soften the cell layers as they were scraped off. After scraping, the scraped portion was carefully cut and the peels were stained in Safranin-O and then mounted in 25% glycerol for examination under the light microscope. Photomicrographs of the epidermis were made for both the adaxial and abaxial surfaces. Trichome type(s), crystal type(s), shapes of the epidermal cells, numbers of epidermal cells, stomata types and stomata frequency were all noted for both leaf surfaces. Also, stomata frequency per square millimeter and stomata index (I) were estimated for the two leaf surfaces using the formula below[16].

$$SI = \frac{S}{(S+E)} \times 100$$

Where 'S' is the number of stomata per unit area; 'E' is the number of ordinary epidermal cell in the same area, "SI" stomata index. The length and breadth of the stomata was measured using ocular micrometer and the measurements were converted to microns using the stage micrometer.

The anatomy of sampled leaflets was studied by cutting transverse sections of the leaflet. All sections were made with the aid of Reichert Sliding Microtome at a thickness of 8-10 microns. The sections were stained in Alcian blue for 3-5 minutes, rinsed thoroughly in water to remove excess stain and counterstained in Safranin O solution for 3-5 minutes. The sections were again washed with water and treated in series of ethanol dilution: 50%, 70%, 80%, 90% and 100% to enhance the dehydration process. The dehydrated sections were transferred into absolute xylene to remove any remaining trace of water and ethanol. These made sections clear and prevented cloudiness of the slide, as well as the drying of the slide. Sections were therefore mounted in 25% glycerol. Photomicrographs of all anatomical features were made with the aid of Accu-scope Trinocular Microscope (Accu-scope 3301 LED Trinocular Microscope with 3.2 MP CMOS digital camera). All measurements were made with the aid of ocular micrometer and final figures derived with ocular constant. For the determination of the cuticle thickness of the leaflets, transverse sectons of the leaves were cut at thickness 20 um using Reichert sliding microtome. Specimens were processed using standard anatomical procedures[17-19]. All microscopic measurements were made with the aid of an ocular micrometer inserted in the eyepiece of the microscope. These measurements were later multiplied by the ocular constant with respect to the objectives under which they were taken.

Data Analysis: 25 micro-anatomical measurements (n=25) were taken for each of the quantitative parameters assessed in order to determine all leaf and petiole micro-anatomical disparity and plasticity in *Triplochiton scleroxylon* in the accessions from the three experimental sites. Data generated were subjected to one-way analysis of variance in a complete randomized design (CRD) using the statistical package for social science (SPSS) version 9.0. Where significant ($p < 0.05$) differences existed, the mean values were separated using the Duncan Multiple Range Test (DMRT) follow-up procedure.

Results and Discussion

Venation pattern of *Triplochiton scleroxylon* leaf: The type of venation pattern in the forty-five (45) *Triplochiton scleroxylon accessions* from the three areas investigated in Akure Forest Reserve was similar, as it was regular polygonal reticulate with a polygonal areolation shape in all (Figure-2). However, the longest areole length, areole width, and interveinal distance were found in the accessions from the Queen's plot, while the least was observed in the accessions from the disturbed forest area. Meanwhile, the accessions from the enrichment plantation have the shortest vein-to-vein distance (Figure-2B, Table-4), which suggests the presence of a more closely spaced venation system[20].

Table-4: Qualitative and Quantitative venational Characters in *Triplochiton scleroxylon* in Queen's plot, Enrichment plantation and Disturbed forest area. Means with the same superscript along the colomn are not significantly different from each other ($p<0.05$).

Site	Venation	Areolation shape	Mean Areole Length (µm)	Mean Areole Width (µm)	Mean Vein Density (µm)	Mean Interveinal Distance (µm)
Queen's Plot	Regular Polygonal Reticulate	Polygonal	352.67 ± 5.21^a	185.34 ± 2.66^a	1.44 ± 0.18^c	102.36 ± 1.84^a
Enrichment Plantation		Polygonal	324.82 ± 4.66^b	178.57 ± 3.40^b	1.69 ± 0.11^b	85.48 ± 0.92^c
Disturbed Forest Area		Polygonal	226.91 ± 3.58^c	139.40 ± 6.12^c	2.10 ± 0.23^a	92.80 ± 1.15^b

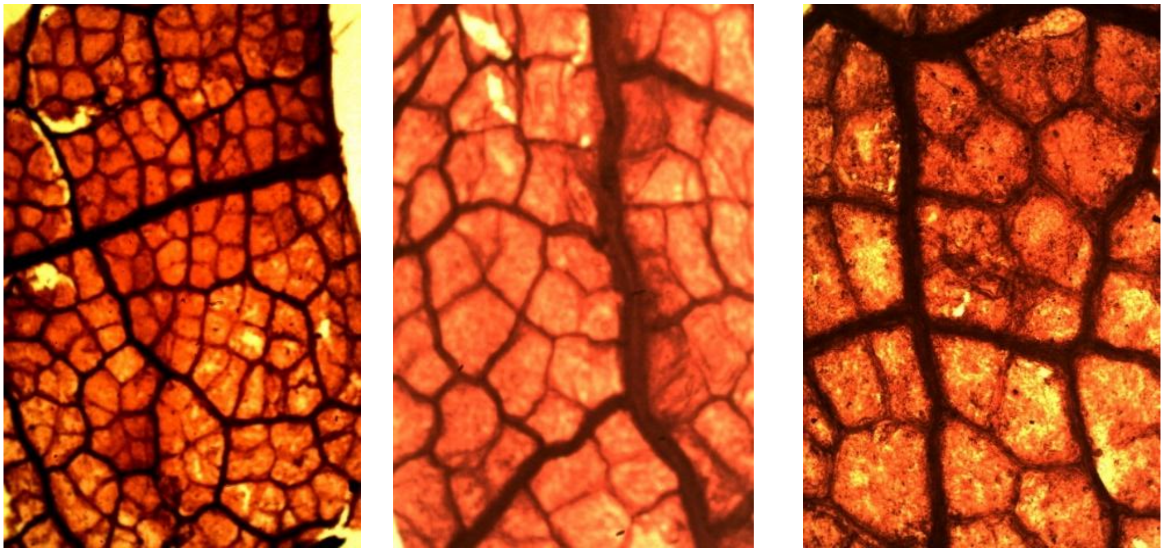

Figure-2: Photomicrographs of venation patterns of *Triplochiton scleroxylon* leaf. A: Venation pattern in Queen's plot accessions, B: Venation pattern in Enrichment plantation accessions, C: Venation pattern in Disturbed forest area accessions.

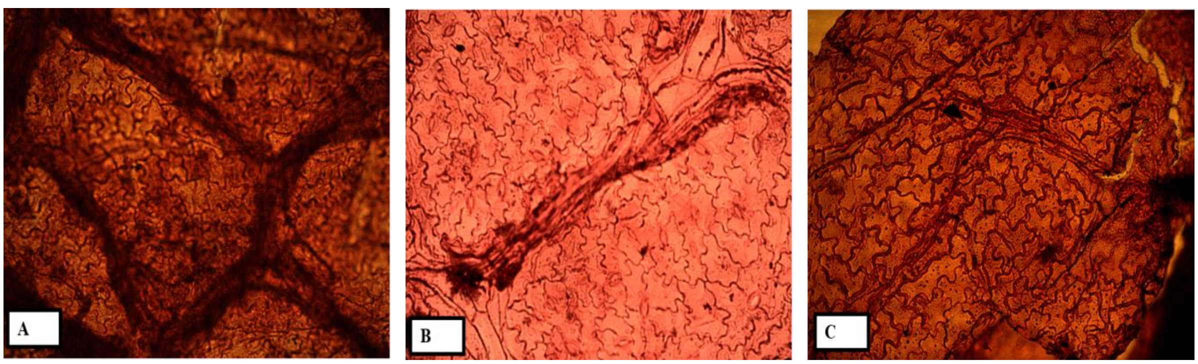

Figure-3: Photomicrographs of Epidermal Studies on adaxial surfaces of *Triplochiton scleroxylon* leaf. **A**: Queen's plot, B: Adaxial surface in Enrichment plantation, **C:** Adaxial surface in Disturbed forest area.

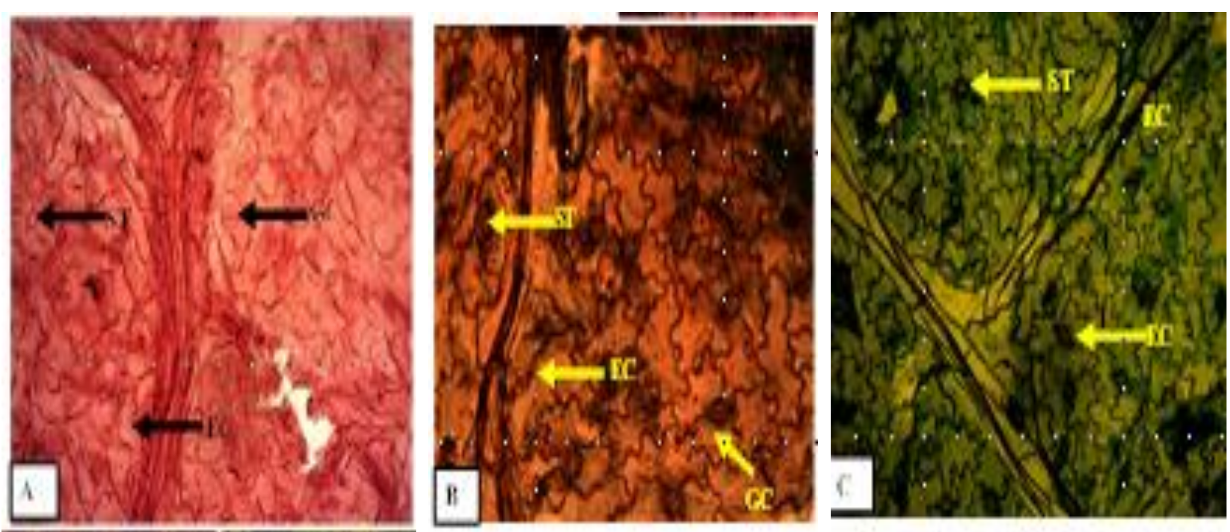

Figure-4: Photomicrograph of Epidermal Studies on abaxial surfaces of *Triplochiton scleroxylon* leaf. A: Adaxial surface in Queen's plot, B: Adaxial surface in Enrichment area, C: Adaxial surface in Disturbed forest area, EC: Epidermal cell, ST: Stomata, GC: Guard cell.

Table-5: Qualitative Leaf Epidermal Micro-anatomy of adaxial and abaxial Surfaces of *Triplochiton scleroxylon* in Queen's plot, Enrichment plantation and Disturbed forest area.

Adaxial Surface						
Land Cover Types	Epidermal cell shape	Anticlinal wall	Periclinal wall	Epidermal papillae	Crystal granules	Trichomes
Queen's plot	Polygonal	Sinuous	Rough	Present, sparse	Present, scanty	Absent
Enrichment area	Polygonal	Slightly sinuous	Rough	Absent	Present, sparse	Absent
Disturbed forest area	Polygonal	Wavy / Slightly sinuous	Rough	Absent	Present, sparse	Absent
Abaxial Surface						
Land Cover Types	Epidermal cell shape	Anticlinal wall	Periclinal wall	Stomata type	Guard cell	Crystal granules
Queen's plot	Polygonal	Wavy	Densely Papillate	Paracytic	Elliptic	Sparse
Enrichment area	Polygonal	Sinuous	Papillate	Paracytic	Crescent	Sparse
Disturbed forest area	Polygonal	Sinuous	Densely Papillate	Paracytic	Crescent	Sparse

Table-6: Quantitative Leaf Epidermal Micro-anatomy of Adaxial Surface of *Triplochiton scleroxylon* in Queen's plot, Enrichment area and Disturbed forest area.

Land Cover Types	Epidermal Cell Density (mm^{-2})	Epidermal Cell Length (μm)	Epidermal Cell Width (μm)
Queen's plot	$147±2.11^{a}$	$42.54±1.52^{ab}$	$27.16±0.29^{b}$
Enrichment area	$111.25±4.56^{c}$	$41.50±2.01^{b}$	$24.90±1.50^{c}$
Disturbed forest area	$138.09±1.24^{b}$	$48.89±1.98^{a}$	$29.53±0.74^{a}$

Mean with the different superscript along the same column are significantly different from each other ($p < 0.05$).

Table-7: Quantitative Leaf Epidermal Micro-anatomical Characters of Abaxial Surface of *Triplochiton scleroxylon*.

Land Cover Types	Queen's plot	Enrichment area	Disturbed forest area
Epidermal Cell Density (mm^{-2})	$67.78±0.29^{ab}$	$52.78±1.11^{c}$	$69.09±0.79^{a}$
Epidermal Cell Length (μm)	$59.78±1.28^{b}$	$59.98±1.84^{b}$	$67.63±1.79^{a}$
Epidermal Cell Width (μm)	$36.58±1.59^{ab}$	$32.29±1.02^{c}$	$37.92±1.27^{a}$
Stomata Density (mm^{-2})	$30.89±0.07^{a}$	$27.48±0.95^{b}$	$24.75±0.73^{c}$
Stomata Length (μm)	$18.25±0.26^{a}$	$10.25±0.25^{c}$	$15.25±0.75^{b}$
Stomata Width (μm)	$10.72±0.63^{a}$	$7.25±0.25^{c}$	$9.75±0.25^{ab}$
Stomata Index (%)	39.12 %	24.94%	29.78%

Means with the same superscript along the row are not significantly different from each other ($p < 0.05$).

Leaf Epidermal Studies of *Triplochiton scleroxylon:* Similarly, all the *Triplochiton scleroxylon* accessions from the three areas investigated possess similar qualitative foliar epidermal characteristics on the adaxial surface, which supports the fact that they are all members of a single species or taxon (Table-5). Meanwhile, epidermal papillae were present only on the adaxial surface of the accessions from the Queen's plot. This anatomical characteristic separated accessions from enrichment plantations and disturbed forest areas from the Queen's plot and can thus be used for the identification of accessions of *Triplochiton scleroxylom* in such areas. However, the absence of stomata on the adaxial surface of all the accessions is taxon-specific (Figure-3A–C). On account of epidermal cells and stomata type and structure, it is worthy of note that the accessions from the three areas are all hypostomatic. The accessions all possess paracytic stomata (Figure-4), polygonal epidermal cell shapes, sparse crystal granules, and elliptic or crescent guard cells. Meanwhile, the wavy anticlinal walls in the accessions from the Queen's plot separate the accessions in the zone from others, as others remain sinuous (Table-5).

Quantitatively, on the adaxial surface, accessions from the Queen's plot have the highest density of epidermal cells, while accessions from the enrichment area have the widest, as the accessions from the enrichment area remain intermediate all through (Figure-4, Table-6). Stomata density, frequency, and distribution are inherently hereditary or genetically controlled traits, but sometimes there may be ecotypes. It was discovered in the study that the higher stomata density and stomata index observed on the abaxial surfaces of the accessions from the Queen's plot (Table-7) suggest that CO_2 could be more readily taken up in the trees from the Queen's plot while being less readily taken up in the accessions from the disturbed forest area. The more stomata per unit area (stomata density), the more CO_2 can be taken up and the more water can be released. This agrees with the findings of Young, J. J. et. al[21].

Leaf Micro-Anatomy (Transverse Sections) of lamina *Triplochiton scleroxylon:* Queen's plot recorded the highest values in epidermal cell density, epidermal cell length, stomata size (length and width), and stomata index (39.12%), although there was no significant difference between the epidermal cell widths on the abaxial surfaces of the accessions from Queen's plot, enrichment plantation, and disturbed area (Table-7). This is related to the higher amount of rainfall that characterizes Akure Forest Reserve. The flaccid and reduced stomata size (stomata length and width) in the accession from the enrichment area is an indication of stomatal closure, which could be related to a conditional reduction in evapotranspiration. Similar reports have been given on the family *Solanaceae*[22-24]. Studies reported that these act as root-shoot signals, eliciting stomatal closure and reducing stomatal conductance and evapotranspiration[25].

For taxonomic and scientific reasons, leaf anatomy offers a number of traits[26,27]. Some researchers have used leaf anatomy for taxonomic consideration in various species of plants, including for genus *Hibiscus*, *Emilia*, and *Celosia*[28-30]. In this study, nearly all leaf micro-anatomical characters encountered are peculiar to all accessions. Such characters include leaves being all bifacial or dorsiventral, uniseriated epidermis, rectangular cells in the epidermal regions, one-layered palisade cells, spindle or oval-shaped spongy cells, collateral vascular bundles in the midrib regions, C-shaped (or crescent) vascular architecture, thin-walled polygonal parenchymatous pith, the presence of simple unicellular trichomes in the epidermal regions, etc. (Figure-5).

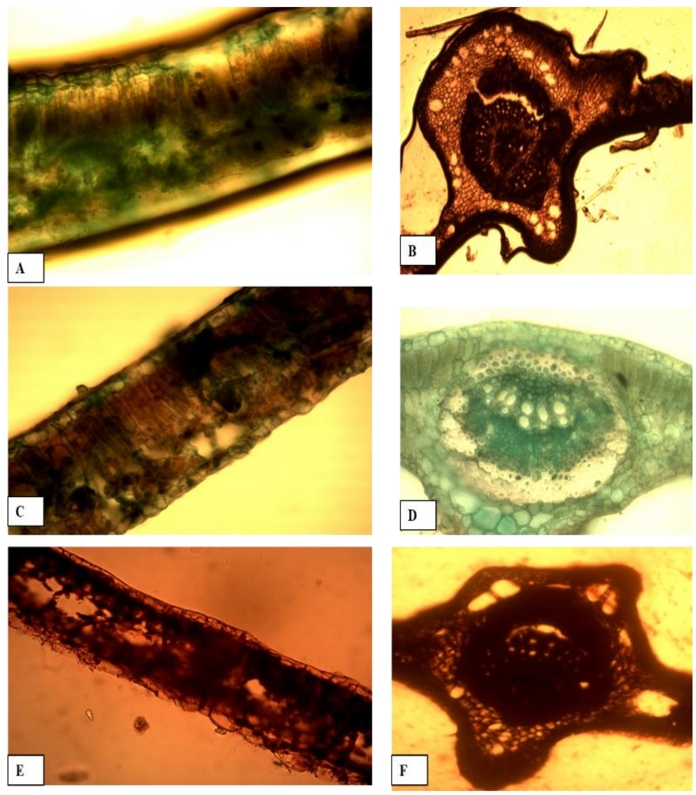

Figure-5: Photomicrograph of the transverse sections of lamina *Triplochiton scleroxylon* leaf. A: lamina of *Triplochiton scleroxylon* from Queen's plot, B: midrib of *Triplochiton scleroxylon* from Queen's plot, C: lamina of *Triplochiton scleroxylon* from Enrichment plantation, D: midrib of *Triplochiton scleroxylon* from Enrichment plantation, E: lamina of *Triplochiton scleroxylon* from Disturbed forest area, F: midrib of *Triplochiton scleroxylon* from Disturbed forest area.

However, the few leaf micro-anatomical variations unveiled could be of great scientific significance. The palisade mesophyll layer in the accessions from the disturbed forest area was thicker than those of the Queen's plot, probably an adaptation to reduce water loss since the disturbed forest area is open unlike the Queen's plot, which has three crowns for covering, while the least was found in the accessions from the enrichment plantation (Table-7). However, the presence of cortical fiber in the midrib region (Figure-5D) of the accession from the enrichment plantation is a spot character.

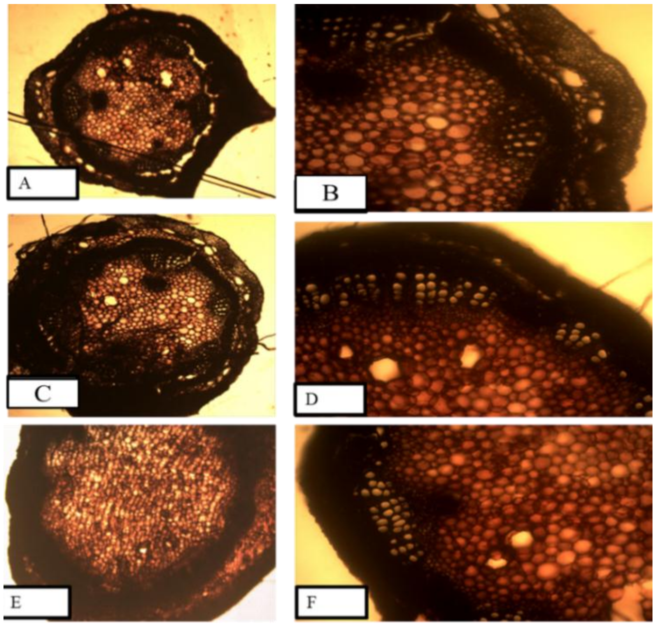

Figure-6: Photomicrographs of Petiole accessions of *Triplochiton scleroxylon* leaf. A and B: Photomicrographs of *Triplochiton scleroxylon* from Queen's plot. C and D: Photomicrographs of *Triplochiton scleroxylon* from Enrichment plantation. E and F: Photomicrographs of *Triplochiton scleroxylon* from Disturbed forest area.

Petiole Anatomy of *Triplochiton scleroxylon:* Data obtained from petiole anatomy can be used for taxonomic and phylogenetic purposes[31]. In the taxon investigated, uniseriate epidermis, angular collenchyma, oval or polygonal parenchyma cells, and pith regions were all found to be specific characters, as they occurred in all 45 accessions (Figure-6 A–F). A slightly rectangular petiole outline delimits accessions from the Queen's plot, while others are circular. Meanwhile, the xylem elements are in compact parallel lines with four to eight cells in each row in all 45 accessions, while the vascular ring is also circular. All these petiole anatomical characters are considered taxon-specific. The type, number, and shape of vascular bundles as adopted in this study were used by in the delimitation of three plant genera: *Croomia*, *Stemona*, and *Stychoneuron* in the family *Stemonaceae*[32]. However, the presence of crystal sands in the parenchymatous pith region in the accessions from the Queen's plot is spot-on.

The significance of epidermal appendages, trichome type, and behavior were critically examined as tools that can be successfully applied when determining petiole micro-anatomical structure and function. Simple unicellular trichomes observed in the epidermal regions of the petioles in all 45 accessions could be the taxon's adaptation to deter through chemical means, as previously reported from previous study which stated that higher trichome densities may be advantageous to plants as they increase resistance against herbivory[33,34]. Previous research also explained the significant influence of trichome types in different organs of the plant body in the delimitation of genera and species of plants[35].

Conclusion

The study on the foliar anatomy of *Triplochiton scleroxylon Queen's* plot, enrichment plantation, and disturbed forest areas in Akure Forest Reserve reveals the micro-anatomical parameters of leaf venation, leaf epidermis, leaf lamina, and petiole are useful and important when researching to discover phenotypic plasticity or phylogenetic relationships in or among plants occupying similar or different habitats.

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