CYTOMORPHOLOGICAL STUDIES ON *HUNTERIA UMBELLATA* AND *PICRALIMA* NITIDA "OSU" OF THE BENIN SPEAKING PEOPLE OF EDO STATE

By

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DEPARTMENT OF PLANT BIOLOGY AND BIOTECHNOLOGY

FACULTY OF LIFE SCIENCES

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BENIN CITY

SEPTEMBER,2023.

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A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF PLANT BIOLOGY AND BIOTECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (HONOURS) DEGREE, IN PLANT BIOLOGY AND BIOTECHNOLOGY.

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CERTIFICATION

This is to certify that this work was carried out by Precious OBAZEE (Miss) of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria.

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DEDICATION

This work is dedicated to GOD Almighty and my family.

ACKNOWLEDGEMENT

I am grateful to God Almighty, who made impossibilities possible for me through guidance, open doors, grace, and constant protection. To my supervisor, Dr. D. I. Omogui I'm grateful for your guidance and inspiration. To my siblings I say a very big thank you for all your invaluable support especially my sister Obazee Blessing. To my Parents Mr and Mrs. Macaulay Obazee, I am highly honoured for all the ways you came through for me. It is indeed a great privilege to have been born by you. I would also like to appreciate all my lecturers in plant biology and biotechnology and most especially the H.O.D Prof D. E. Vwioko and Dr. J. O. Erhabor for their endless support and advice. To Mr. Fetus I specially appreciate you for your invaluable efforts, guidance, and support during the course of this project. To my roommate Itoyah Blessing, I want to thank you for your endless support, care and advice, God bless you. To my friends especially Omoteregha Ebruphihor, Nosayakiodehia Uduebor and Okpavuerhe O. Peace, I'm highly indebted for their support. God bless you all. Above all I give God the glory for his grace, mercies, direction, protection and guidance on this work, you saw me through it all.

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ABSTRACT

This research aimed to investigate the cytomorphological aspects of *Picralima nitida* (Stapf) T. Durand & H, Durand and *Hunteria umbellata* (K.Schumann) H. Hallier, both belonging to the Apocynaceae family, within Edo State. The objectives included studying cytological details, potential evolutionary rates, and distinguishing differences between the two species. Matured plant samples were collected from the University of Benin's botanic garden and various locations in Benin City. Morphological characteristics, leaf length and width, and plant height were examined. Cytological techniques involved observing meiosis in flower buds and obtaining epidermal peels for microscopic analysis. The study revealed various stomatal types and their distribution, contributing to taxonomic insights. Chromosome numbers were assessed, with 2n=22 confirmed for *Picralima nitida*. The chromosome count for *Hunteria umbellata* couldn't be confirmed due to pollen production interference. The constancy of chromosome numbers was emphasized as a crucial species trait. Epidermal characteristics, including cuticle, shape of subsidiary cells, and stomatal wall patterns, were explored for taxonomic and systematic significance. Stomatal abnormalities, such as contiguous stomata in *Hunteria umbellata*, were observed, suggesting ongoing evolutionary processes.

CHAPTER

1.0 INTRODUCTION

Apocynaceae (from Apocynum, Greek "dog-away") is a family of flowering plants that includes trees, shrubs, herbs, stem succulents, and vines, commonly known as the dogbane family (Endress and Bruyns, 2000), because some taxa were used as dog poison (Simpson and Michael, 2010). Apocynaceae consists of 424 genera with more than 4,600 species distributed in five subfamilies such as Rauvolfioideae, Apocynoideae, Periplocoideae, Secamonoideae, and Asclepiadoideae Members of the family are native to the European, Asian, African, Australian, and American tropics or subtropics, with some temperate members (Endress and Bruyns, 2000). The former family Asclepiadaceae (now known as Asclepiadoideae) is considered a subfamily of Apocynaceae and contains 348 genera. A list of Apocynaceae genera may be found here (Simpson and Michael, 2010). Many species are tall trees found in tropical forests, but some grow in tropical dry (xeric) environments. Also perennial herbs from temperate zones occur. Many of these plants have milky latex, and many species are poisonous if ingested, the family being rich in genera containing alkaloids and cardiac glycosides, those containing the latter often finding use as arrow poisons. Some genera of Apocynaceae, such as *Adenium*, bleed clear sap without latex when damaged, and others, such as *Pachypodium*, have milky latex apart from their sap.(Michael*, et al.,* 2012).

A characteristic feature of the family is that almost all species produce milky sap.

Leaves are simple, opposite and whorled. Flowers are large and colourful. The flowers are bisexual and actinomorphic or sometimes weakly zygomorphic. The calyx is synsepalous and usually 5-lobed. The corolla is sympetalous and usually 5-lobed. The stamens are distinct, as many as corolla lobes and alternate with them, and adnate to the corolla tube (or perigynous zone). The anthers are turned inward and commonly adherent to the surface of the stigma. The gynoecium consists of a single compound pistil of 2 carpels that may be distinct at the level of the superior or rarely partly inferior ovary but which are joined together by a single style. When distinct, each ovary typically has few to numerous ovules on marginal placenta; when connate, the placentation is axile or intruded parietal. A nectary consisting of 5 glands or an annular ring is usually found at the base of the ovary. The fruit is commonly a follicle, capsule, or berry. The seeds are mostly flat and winged or have a clump of hairs at one end. The Apocynaceae family has significant ethnobotanical importance, as many of its genera and species have been traditionally used by various cultures for medicinal, cultural, and practical purposes. There are common ethnobotanical uses of plants from the Apocynaceae family which are; for medical uses, for fiber uses, as an ornamental plant etc. Traditional societies over the years have employed medicinal plants in ethno-medicine for the treatment of various diseases without the help or knowledge of scientific method of the physiologically active ingredients called phytochemicals which were responsible for the plants' medicinal and pharmacological potentials (Aja *et al*., 2010; Akubugio *et al.*, 2007; Adimoelja, 2000).

Scientifically Apocynaceae can be classified as;

Kingdom Plantae

Division Angiosperms

Class Eudicots

Subclass Asterids

Order Gentianales

Family Apocynaceae

The subfamilies include:

Rauvolfioideae

Apocynoideae

Periplocoideae

Secamonoideae

Asclepiadoideae

A typical and prominent species of the Apocynaceae is *Picralima nitida* and *Hunteria umbellata.*

1.1 *Picralima nitida* ((STAPF) T.DURAND and H.DURAND)

Picralima nitida is the only species of the genus *Picralima* and it is related to *Hunteria* and *Pleiocarpa*. *P. nitida* (Syn. *Picralima klaineana* Pierre) is commonly called Picralima, Akuamma or Pile plant, it belongs to the hunterieae tribe of the apocynaceae family. The Yoruba calls it Abeere (*Picralima nitida*). It is known as Osu

in Edo. Another plant, *Hunteria umbellata* is also called Osu, though the poison principles in all plant parts makes it something that must be used with caution. The plant is widely distributed in high deciduous forest of West-Central Africa from Ivory Coast to West Cameroons, and extending across the Congo basin and Uganda (NNMDA, 2008). *P. nitida* is an understorey tree which reaches up to 4 - 35 m in height, crown dense, trunk 5-60 m diameter; cylindrical, the wood is pale yellow, hard, elastic, fine-grained and taking a high polish. *P. nitida* bears white flowers (about 3 cm long) with ovoid fruits which at maturity are yellowish in colour. The leaves are broad (3-10 cm) and oblong (6-20 cm long) with tough tiny lateral nerves of about 14 to 24 pairs Burkill (1985). *P. nitida* has widely varied applications in West Africa folk medicine. Various parts of the plant; the leaves, seeds, stem bark and roots are used by herbalists for the treatment of fever, hypertension, jaundice, gastro-intestinal disorders and for malaria Etukudo, (2003). The extract from different parts of the plant have been found to exhibit a broad range of pharmacological activities which lends acceptance to its ethnomedicinal uses. Indole alkaloids isolated from the seeds of *P. nitida* such as akuammine, akuammidine, akuammicine, akuammigine and pseudoakuammigine are interesting compounds with opoiod analgesic activity. The pharmacological potential of these alkaloids have only partially been investigated, therefore more research is to be done to completely explore their pharmacologic and therapeutic potentials.

1.1.1 Botanical description of *Picralima nitida*

The Akuamma tree (*Picralima nitida)* is a medium-sized evergreen tree that can grow

up to 15-35 meters in height. Shrub or tree up to 35 m tall, with white latex in all parts, glabrous; bole up to 60 cm in diameter; bark hard, brittle, pale to dark grayish black or brown, smooth to slightly rough or finely striped. Leaves opposite, simple and entire; stipules absent; petiole 1–2 cm long; blade elliptical to oblong, (5–)10–26 cm × 2–13 cm, base cuneate, apex abruptly acuminate, thickly papery to thinly leathery, pinnately veined with 14–23 pairs of lateral veins. Inflorescence a terminal or sometimes axillary, compound, umbel-like cyme 6–10 cm long, 10–35-flowered; peduncle 2–35 mm long, with 3 primary branches; bracts very small. Flowers bisexual, regular, open during the day; pedicel 2–20 mm long; sepals almost free, imbricate, broadly ovate to almost orbicular, 5–7 mm long; corolla with fleshy cylindrical tube 25–45 mm long, hairy inside and narrowed below the insertion of the stamens, often greenish, lobes ovate, 14–30 mm × 6–10 mm, apex obtuse, spreading or erect, white to yellow; stamens inserted above the middle of the corolla tube, included, anthers ovate, 3–4 mm long; ovary superior, consisting of 2 separate carpels, united at the extreme base by a disk-like thickening, style slender, 5–7 mm long, pistil head with an oblong basal part and a filiform stigmoid apex up to 1.5 mm long. Fruits consisting of 2 free obovoid to ellipsoid follicles 11–20 cm long, smooth, apex rounded, yellow to orange, 2-valved, several- to many seeded. Seeds obliquely ovate, obovate to oblong, flattened, 2.5–4.5 cm long, smooth, brown to orange, embedded in soft white to orange pulp. Seedling with epigeal germination; cotyledons ovate to obovate or oblong, 10–13 mm long, base slightly cordate to rounded, apex obtuse to rounded.

1.1.2 Distribution and habitat of *Picralima nitida*

Picralima nitida is primarily found in the following countries in West Africa: Nigeria, Ghana, Ivory Coast, Cameroon, Gabon, Republic of Congo, Democratic Republic of the Congo (DRC). The Akuamma tree (*Picralima nitida*) is typically found in tropical and subtropical regions of West Africa. It prefers to grow in well-drained, sandy-loam soils and can often be found in lowland rainforests, riverbanks, and open savannas.

1.1.3 Ethno-botanical uses of *Picralima nitida* in West Africa

In Gabon, the seeds are applied externally for the treatment of abscesses. In Ghana, the seed-decoction is given as an enema while the crushed seed is taken by mouth for chest-complaints, pneumonia, gastrointestinal disorders and dysmenorrhea. In Ghana, the fruit's hard shell is filled with palm-wine which is drunk after it has absorbed the bitter principle and it's taken as a remedy for fever. The leaves are used as a vermifuge and the leaf-sap is dripped into the ears for otitis. The bark is used as laxatives and purgative, anthelmintic, treatment of venereal diseases, febrifuges and also to treat hernia. In Ivory Coast, a decoction of the bark is drunk in drought for jaundice and yellow fever. The root is used as vermifuge, aphrodisiac and anti-malaria (Faluyi,2021).

1.1.4 Ethno botanical uses of *Picralima nitida* in Edo state

In Edo State, Nigeria, *Picralima nitida,* commonly known as Akuamma, but known to the Benin speaking people as Osu and holds significant ethnomedicinal importance. The plant has been utilized by the indigenous communities for its various medicinal properties. Osu seeds are traditionally used as a natural painkiller. Osu has been

employed as a digestive aid, assisting in the relief of gastrointestinal issues such as stomachaches and indigestion. Osu seeds are sometimes used in cultural and traditional ceremonies in Edo State. It is deeply rooted in traditional medicine practices and is considered a valuable natural resource for promoting health and well-being. The plant's traditional uses have been passed down through generations, contributing to the cultural identity and heritage of the local communities. It's important to note that while *Picralima nitida* has a long history of traditional use, scientific research is ongoing to explore its pharmacological properties and validate its traditional claims.

The seeds of *Picralima nitida* have been traditionally used for various ethno-botanical purposes, particularly in traditional medicine. Traditional use suggests that the seeds may have properties that could be beneficial for managing diabetes. The stem bark, fruit and seeds contain a number of indole alkaloids. Preparations from different parts of the plant are used in traditional medicine for the treatment and management of malaria, abscesses, hepatitis, pneumonia, diabetes, hypertension, pneumonia and other chest-conditions. The seeds (they are hard) are usually ground to a fine powder and added to foods such as ogi (pap) or taken as an infusion (as if making tea). Some take it with water while some soak the seeds or its powder in coconut water and drink. (Faluyi,2021).

1.1.5 Scientific classification of *Picralima nitida*

Kingdom: Plantae

Phylum: Tracheophytes

Class: Magnoliopsida

Order: Gentianales

Family: Apocynaceae

Subfamily: Rauvolfioideae

Tribe: Hunterieae

Genus: Picralima /Pierre

Species: *Picralima nitida*

1.2. *Hunteria umbellata* ((K.SCHUM.) HALLIER F.)

Hunteria umbellata grows as either a shrub or tree up to 22 metres (72 ft) tall, with a trunk diameter of up to 40 centimetres (16 in). Its flowers feature a white, creamy or pale yellow corolla. The fruit is yellow and smooth. Its habitat is forests from sea level to 600 metres (2,000 ft) altitude. Its numerous local medicinal uses include for fever, leprosy sores, stomach and liver problems and as an anthelmintic, especially against internal worms. Hunteria umbellata has been used as arrow poison. The plant's hard wood is used in carving and to make small tools (PROTA, 2008). The species is native to an area of tropical Africa from Guinea-Bissau in the west to Angola in the south. (IUCN, 2019).

1.2.1 Botanical description of *Hunteria umbellata*

Shrub or small tree up to 15(–22) m tall, with colourless or milky latex in all parts; bole sinuous or straight, up to 40 cm in diameter, fluted; outer bark 1 mm thick, rough or smooth, grey to dirty brown; crown dense. Leaves opposite, simple and entire; stipules absent; petiole 1–2.5 cm long; blade elliptical to oblong, up to 22.5 cm × 11

cm, cuneate to obtuse at base, obtuse to acuminate at apex, glabrous, leathery, pinnately veined. Inflorescence a terminal, rarely axillary, dense to lax cyme, 10–20(–80)-flowered; peduncle up to 1.5 cm long. Flowers bisexual, regular, 5-merous, fragrant; pedicel 3–7 mm long; sepals almost free, broadly ovate to triangular, 0.5–2 mm long, erect and stuck to the corolla tube with thick resinous substance; corolla white, creamy or pale yellow, with cylindrical tube 4–8 mm long, lobes 6–12 mm long, twisted in bud, with a belt of hairs inside the tube just below the insertion of the stamens; stamens inserted in the upper part of the corolla tube; ovary superior, composed of 2 separate carpels, abruptly narrowing into the style, terminating in a stigmatic ellipsoid basal part and a 2-lobed apex. Fruit consisting of 2 separate globose mericarps 3–6 cm long, yellow, smooth, 8– 25-seeded. Seeds oblong to ellipsoid, 1–1.5 cm long, flattened at one side.

1.2.2 Distribution and habitat of *Hunteria umbellata*

Hunteria umbellata native to most region in West Africa countries like Nigeria, Ghana, Sierra Leone, Ivory Coast, and others in the region. *Hunteria umbellata* is commonly found in the tropical rainforests and moist, deciduous forests of West Africa. It thrives in regions with a humid and warm climate, where it receives adequate rainfall. The tree prefers well-drained, fertile soils, but it can tolerate a range of soil types.

1.2.3 Ethno botanical uses of *Hunteria umbellata* seed in Edo state

In Edo state Nigeria, *Hunteria umbellata* is commonly known as “Africa yam pepper” in Nigeria but know to Benin speaking people as Osu. *Hunteria umbellata* (Osu) has a long history of medicinal use among the indigenous people of Edo State. Different

parts of the plant, such as the seeds, leaves, and roots, are use in traditional medicine for various purposes. It is believed to possess medicinal properties and is used to treat ailments like stomach disorders, fever, malaria, and other conditions. In some culture within Edo State, *Hunteria umbellata* may also have ritualistic or ceremonial significance. It could be used as part of traditional rituals, cultural ceremonies, or spiritual practices. The plant may also have economic importance, as communities could engage in trade or local commerce involving the harvesting, processing, and selling of *Hunteria umbellata* or its products. Like many other indigenous plants, *Hunteria umbellata* is likely to have cultural significance, being part of the local knowledge and traditional practices passed down through generations. Specific details and cultural associations may vary among different communities and regions within Edo State.

In the regions where Hunteria umbellata is found in Edo state, the seeds are sometimes used for various purposes. In traditional medicine, *Hunteria umbellata* seeds are believed to have medicinal properties. They may be used to treat various ailments, such as digestive issues, coughs, and as a general tonic. However, it's crucial to note that traditional medicinal practices can vary, and the effectiveness and safety of such uses would require scientific approval. While the fruit is mainly consumed for its juicy pulp, the seeds might be use for cooking. They could be ground into a flour and used as a thickener in soups or sauces, similar to other seeds and nuts in some African cuisines. Hot and cold decoctions made from the plant seeds have also been reported to be highly valued in the local treatment of obesity, hypertension, pain and

swellings, anaemia and as immune booster (Boone, 2006; Adeneye and Adeyemi, 2009).

1.2. 4 Scientific classification of *Hunteria umbellata*

Kingdom	Plantae (Plants)
Clade	Tracheophytes (Vascular plants)
Clade	Angiosperms (Flowering plants)
Clade	Eudicots (True dicotyledons)
Clade	Asterids
Order	Gentianales
Family	Apocynaceae
Genus	Hunteria
Species	*Hunteria umbellata*

1.3 CYTOLOGICAL STUDIES

Cytotaxonomic or cytogenetic or cytology studies provide taxonomic insights, at different hierarchical levels, not only through the determination of chromosome numbers but also through the development and comparison of karyotypes. Comparative karyotype analysis of related species has been used to describe patterns of chromosomal evolution and variation within a group. This field has enabled researchers to better understand the mechanisms of inheritance, crop improvement and the discovery of genes that are important to plant health. Cytology has also enabled scientists to better understand the physiology of plant-environment interactions, as well as to make more informed plant selection decisions.

1.4 AIMS AND OBJECTIVES

The aim of this research is to investigate the cytomorphological studies of selected species of Apocynaceae (*Picralima nitida* and *Hunteria umbellata*) collected within Edo state

The objectives of this studies were to:

I. Investigate their cytological information and determine the rate of evolution if possible; and

II. Assess the differences between the two species where possible

CHAPTER TWO

2.0. MATERIAL AND METHODS

2.1 SOURCE OF PLANT MATERIAL

Matured shoot of the plants were collected from the PBB Department botanic garden in the University of Benin, Benin City, Edo State. *Picralima nitida* was examined in their cultivated habitats. Parts of live plants used for this study were also collected from different parts of Benin City. The plants were then transplanted to experimental pots filled soil.

2.3 MORPHOLOGICAL CHARACTERISTICS

The morphological characteristics of the buds, stem, leaves, its length and width with the height of each species was examined.

2.4 MEASUREMENT OF THE LENGTH AND WIDTH OF THE LEAVES

The length and width of fifty leaves each of the two species were measured using a meter rule.

2.5 HEIGHT OF PLANT SPECIES

The heights of the different species were measured using measuring tape and subsequently recorded.

2.6 CYTOLOGICAL TECHNIQUES

a) Schedule of Meiosis

This was carried out according to MC Clintock's method. Young flower buds of different sizes were collected and fixed in acetic acid: ethanol: chloroform (1:3:6)

mixture for 12 hours and later transferred to 70% alcohol (ethanol as the fixative). The anthers were dissected out from the flower buds and placed in a slide (clean slide), a glass rod was used to squash the anther in 2% aceto-carmine to release the contents, and the debris were removed using forceps. A drop of 2% aceto-carmine were added to it. A cover slip was placed on it, the slide was slowly moved over the spirit lamp to warm the anthers and then place in between filter papers and a little pressure was exerted on the slide and examined under the microscope (Johansen, 1940).

2.6 TECHNIQUE FOR OBTAINING EPIDERMAL PEELS

Epidermal observations was carried out on fresh material. Sample of fresh material were taken from identical regions of the abaxial and adaxial surface of each leaf, usually from midway between the base and the apex of the lamina. Epidermal peels were obtained following the method of Gill and Nyawuame (1990).

2.7 SCHEDULE OF FOLIAR SPECIMEN

Epidermal peel was obtained by peeling off a section using a pair of forceps. Later it was bleached and washed up with tap water. The bleached material was mounted on the slide after washing up with distilled water. A Carmel hairbrush was used to clean the bleached materials until the stomata were visible. Clear epidermal structure was then seen under the microscope.

2.8 VIEWING PREPARED SLIDE USING LIGHT MICROSCOPY

Slides for all the samples of fresh foliar materials were examined under a light microscope, using x10 eye piece and ×40 objective lens (×400) for uniformity. Key information were noted and recorded.

2.9 PHYTODERMOLOGICAL STUDIES

Epidermal cell, stomatal distribution, morphology of mature stomata and nature of stomatal abnormalities were observed. The terminologies for description of mature stomatal adopted were that of Rasmussen (1981) and Vancothem (1970).

2.10. Stomata Terminology

Drawings depicting the observed stomata types are shown in Figure 1.

TYPE A: Anomocytic (Ranulaceous) type: Stomata surrounded by neighbouring cell that are in distinguishable in size or form the remaining epidermal cells.

TYPE B: Actinocytic (pericytic) type: Stomata surrounded by a circle of radiating subsidiary cells.

TYPE C: Anisocytic (unequal-celled, cruciferous) type: Stomata surrounded by subsidiary cells of which one is distinctly smaller than the other two.

TYPE D: Bicytic-paracytic (parallel celled, Rubiaceous) type: Stomata surrounded on either side by one or more subsidiary cells parallel to the long axis of the pore and guard cells.

TYPE E: Bicytic-diacytic (cross celled, labiatous, caryophyllaceous) type: Stomata surrounded by a pair of subsidiary cells whose common wall is at right angle to the guard cells.

Type F: Tetracytic (Monocotyledonous) type: Stomata surrounded by four subsidiary cells (two lateral and two polar)

Type G: Hexacytic (Monocotyledons) type: Here, stomata are surrounded by six

subsidiary cells (four laterals and two polar)

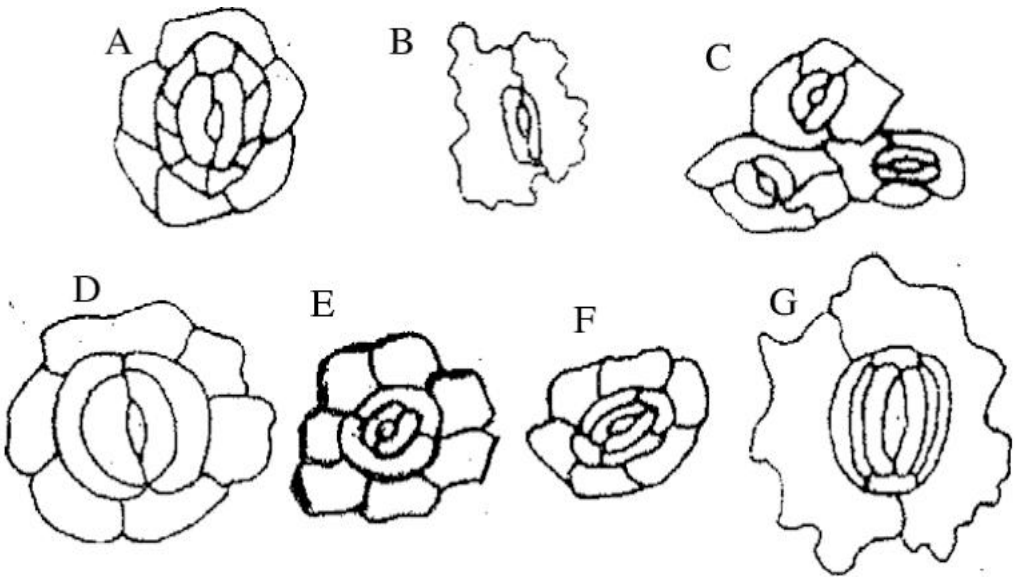

Figure 1: Stomatal Terminology Drawings

A = Anomocytic

B = Actinocytic

C= Anisocytic

D= Bicytic-Paracytic

E= Bicytic-Diacytic

F= Tetracytic

G= Hexacytic

(Rasmussen, 1981)

2.11 Morphology and terminology of tricolpate and related pollen grains.

Drawings depicting reported Pollen types are shown in Figure 2.

A=Abies (Tschundy and Scott 1969)

B= Pinus (Traverse, 1988)

C= Tsuga (Traverse, 1988)

D= Podocarpus (Tschundy and Scott 1969)

E= Aquilapollenites (Traverse 1988)

F= Woodhousia (Traverse, 1988)

G= Picea (Moore *et al*., 1991)

H= Alnus (Moore *et al*. 1991)

I= Betula (Moore *et al*., 1991)

J= Carpinus (Moore *et al*. 1991)

K= Acer (Moore *et al*., 1991)

L= Quercus (Moore *et al*., 1991)

M= Corylus (Moore *et al*., 1991)

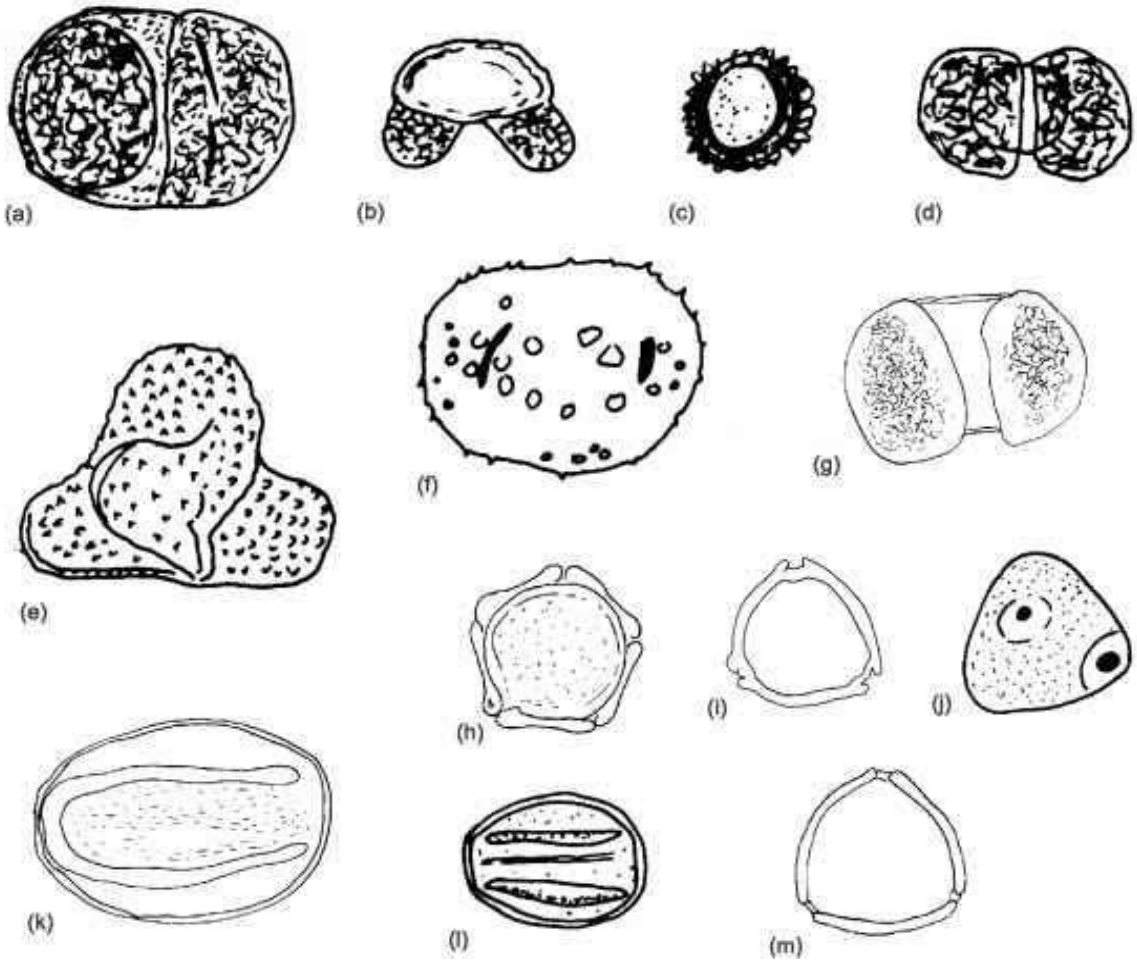

Figure 2: Pollen Terminology Drawing

CHAPTER THREE

3.0 RESULTS

3.1 Ethno description of the selected species

Two species under the family Apocynaceae have been identified. The two species are commonly known to the Benin speaking indigenous people as Osu. The local name 'Osu' of the Benin speaking people have been found to be used to describe two different species that are morphologically alike and they have been identified at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State. The choice of this plant was based on their morphological similarity and to record any cytological differences in the two species. The locality of the species has been summarized in Table 3.1.

Table 3.1: Locality of the two species (*Picralima nitida* and *Hunteria umbellata)*

State	City	Community
Edo-State	Benin-City	Ugbowo, TV road
Oyo- State	Ibadan- City	Idi-Igea Village, Fapoti Olowode Village.

Plate 3.1: *Hunteria umbellata*

Plate 3.2: *Picralima nitida*

3.1 MORPHOLOGY RESULT

a) *Hunteria umbellata* ((K.SCHUM.) HALLIER F.)

HABIT: This type was observed to be a tree. Normal terrestrial woody plant.

HEIGHT: 22 metres (72 ft) tall

FRUIT: Dark green, smooth and oval shape

LEAVES: Cuneate to obtuse at base, obtuse to acuminate at apex, smooth and glossy, light green

EPIDERMAL MORPHOLOGY: Epidermal cells are indistinguishable in size, both surfaces are straight

STOMATA SHAPE: Bicytic-paracytic

b) Picralima nitida ((STAPF) T.DURAND and H.DURAND)

HABIT: This is an evergreen tree or shrub, dark green, with a lot of leaves

HEIGHT: 15- 35 meters tall

FRUIT: Smooth, rounded, yellow to orange

LEAVES: base cuneate, apex abruptly acuminate, thickly papery to thinly leathery, dark green

EPIDERMAL MORPHOLOGY: Epidermal cells of both surfaces are straight

STOMATA SHAPE: Anomocytic

Table 3.2: Biometric measurement of the leaves of the two species

Species	Length(cm)	Width (cm)
Picralima nitida	18.10 ± 0.84	7.50 ± 0.48
Hunteria umbellata	17.19 ± 1.46	7.22 ± 0.83

3.2 CYTOLOGICAL RESULT

Chromosome number: From the meiosis studies, the chromosome number of *Picralima nitida* was found to be 2n= 22 and for the *Hunteria umbellata*, it was found to be very difficult with millions of pollen grains always produced from the flower bud even when the bud has not opened.

Table 3.3: Epidermal Morphological Characters of the two species of Apocynaceae (*Picralima nitida and Hunteria umbellata)*

Species	**Habit**	**Nature of material**	**Leaf surface**	**Wall pattern**	**Morphological type of stomata**	**Trichrome**
Picralima	Tree or	Fresh	U	Straight	Anomocytic	Absent
nitida	shrub		L	Straight	Anomocytic	Absent
Hunteria	Shrub	Fresh	U	Straight	Bicytic –paracytic	Absent
umbellata	or tree		L	Straight	Bicytic -paracytic	Absent

U = upper (adaxial), L = lower (abaxial)

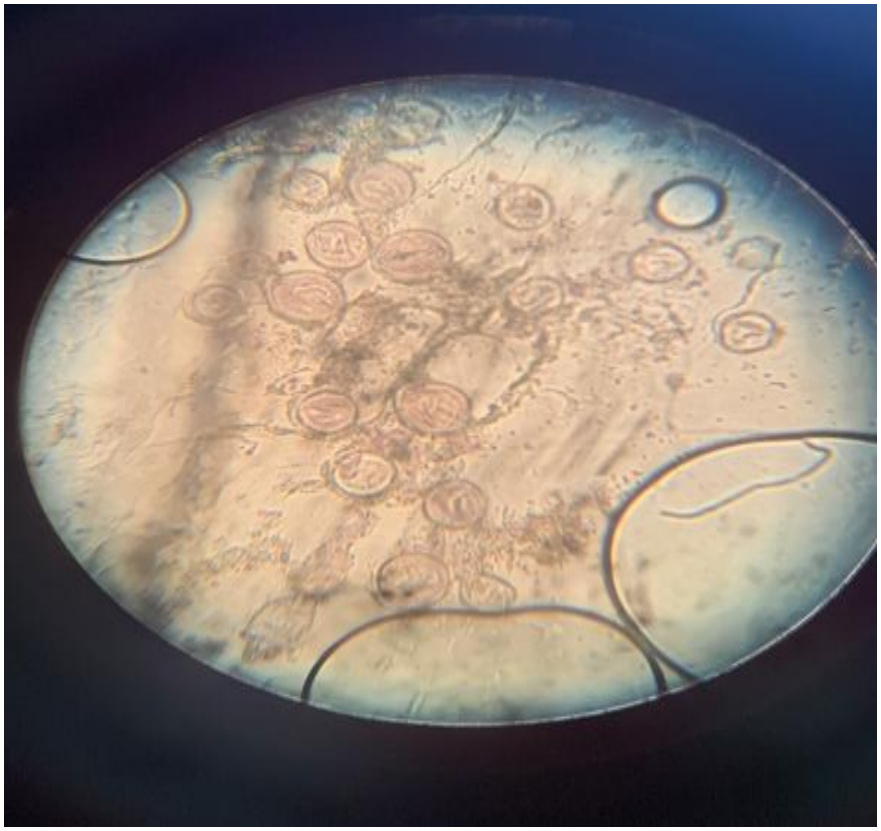

Plate 3.3: Pollen grain of *Picralima nitida*

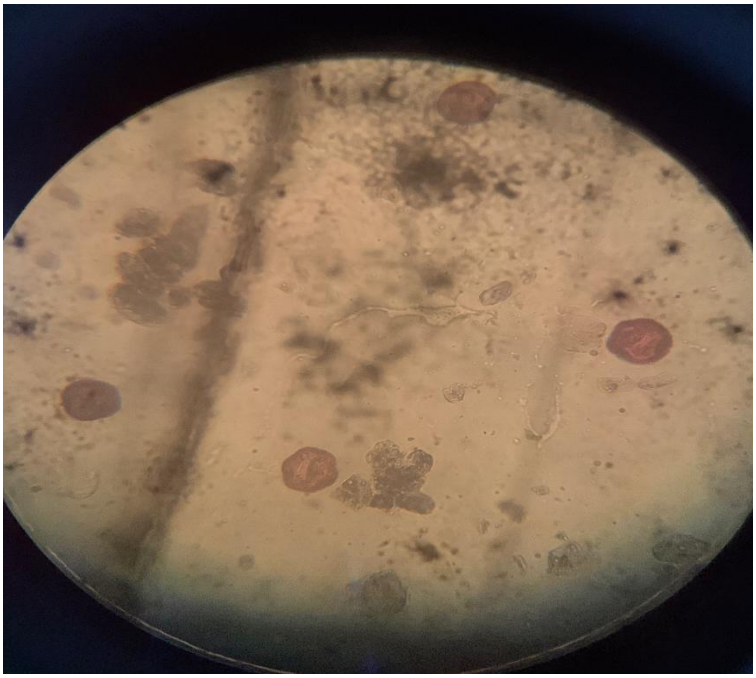

Plate 3.4: Pollen grain of *Hunteria umbellata*

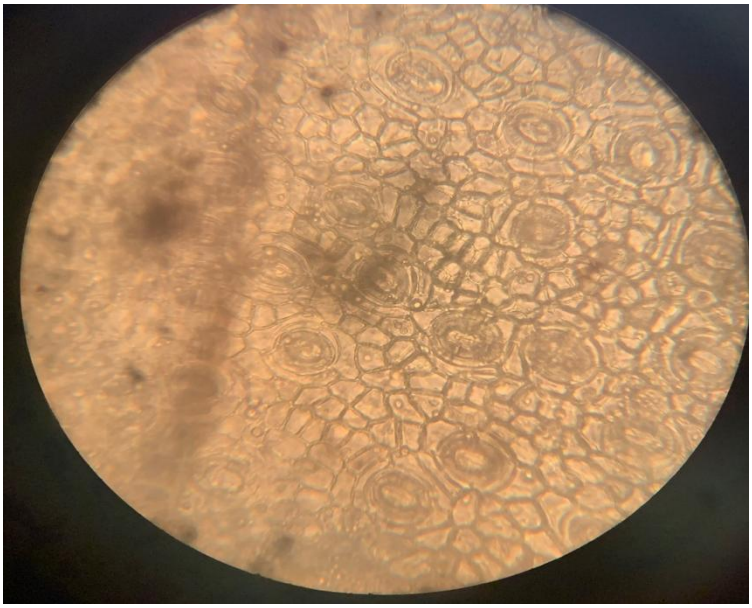

Plate 3.5: Stomata of *Picralima nitida* (Abaxial)

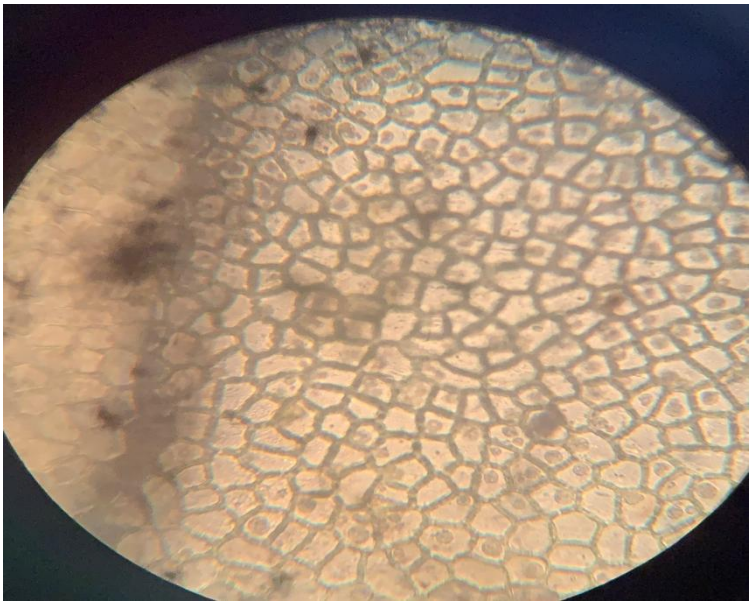

Plate 3.6: Epidermis of *Picralima nitida* (Adaxial)

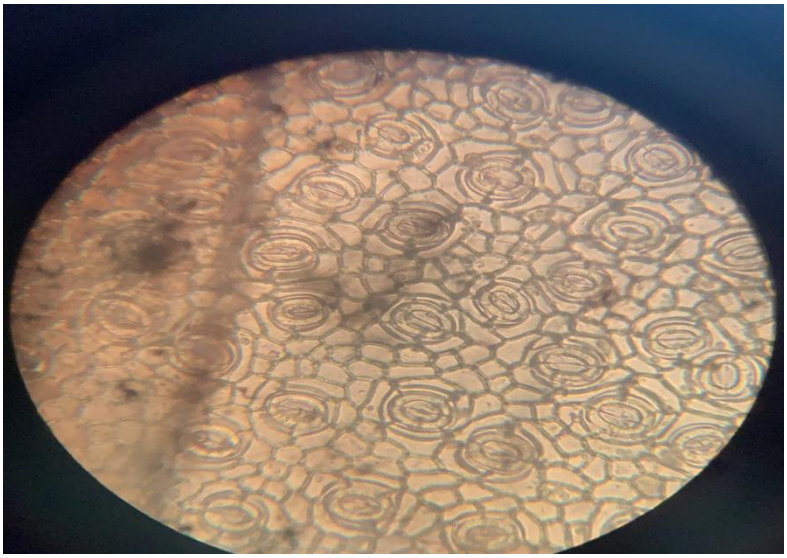

Plate 3.7: Stomata of *Hunteria umbellata* (Abaxial)

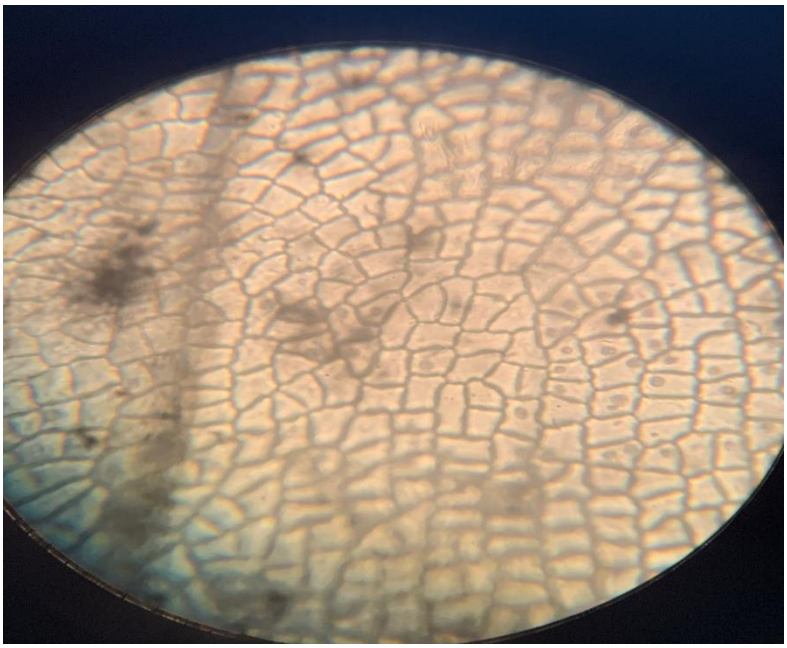

Plate 3.8: Epidermis of *Hunteria umbellata* (Adaxial)

Plate 3.9: Chromosome number of *Picralima nitida* (2n=22)

Plate 3.10: Tetrad formation in *Picralima nitida*

Plates 3.3 – 3.4 show photos of pollen taken under the microscope:

The Pollen of *Picralima nitida* was an Acer pollen while that of *Hunteria umbellata* was an Alnus pollen.

Plates 3.5 – 3.8 show photos of the upper and lower epidermis of both species:

Stomata was observed on the lower epidermis and not on the upper epidermis.

Plate 3.9 – 3.10 show photos of the chromosome and tetrad of *Picralima nitida*

CHAPTER FOUR

4.0 DISCUSSION

4.1 CHROMOSOME NUMBER

Chromosome number have been widely used in systematic investigation in the plant kingdom. For example, King, *et. al*. (1976) divided the tribe Eupatoriceae into four sub-tribes on the basis. Olowokudejo and Heywood (1984) used chromosome number in solving the taxonomic difficulty in the genus *Biscutella*. The information on chromosome number when used in conjunction with other taxonomic data tends to provide a better understanding of the tribal and generic relationship within the plant kingdom (Jansen and Stuessy, 1980).

The count of 2n = 22 for *Picralima nitida* in Plate 3.9, agrees with NyunaÏ and Njifutié (2011) chromosome count for the species. The chromosome number of *Hunteria umbellata* could not be confirmed because of the high production of pollen grains from their bud. At present there is karyological information about 10% of the species and about 30% of the genera of the Apocynaceae. Basic numbers of n= 6, 8, 9, 10, 11,12, 16, 18, 20, 21 and 23 have been assessed. Of these n = 11 is primitive, occurring in about 60% of the genera (Van der Laan and Arends, 1985). Most genera of the Africa continent, which are well known regarding their chromosome number are characterized by n= 11. Proving the chromosomal count found in *Picralima nitida.*

The constancy of chromosome number is an important property of the species because any change in chromosome number results in loss or addition of genes with the consequent disturbance of the delicate physiological balance within the affected

species Haskell and Wills, (1986). Many workers have observed this difficulty in maize. Early workers like Duncan and Ross (1950) and Punnett (1953) have attributed this difficulty of getting the maize chromosome to the high accumulation of calcium oxalate in the ovule. This accumulation of calcium oxalate starts from 10-12 days after pollination while Chattopadhyay and Sarkar (2005) have attributed it to the insensitive nature of the endosperm to most of the common treatment used in chromosome studies as such promoting endomitosis and this makes chromosome count to be erroneous.

4.2 EPIDERMAL MORPHOLOGY

The cuticle of the angiosperm leaf is an easily studied characters and therefore very important in the intra-generic systematic and phylogeny. Epidermal characteristics of plants have proved to be a very reliable taxonomic character be a very reliable taxonomic character after cytology and it is very useful in paleobotany for the identification of fossil leaf impressions in peat stratigraphy and animal fodder research medicine Gill and Nyawuame (1990).

The works of Stace (1965), Wilkinson (1979) and Dehgan (1980) have shown the value of epidermal characters' s taxonomic evidence when used in conjunction with other data can result in sound taxonomic decisions. Metcalfe (1954) pointed out that certain characters such as micro hair, shape of subsidiary cells of the stomata, wall pattern and silica bodies are important systematically. The stomata and epidermal characters might be used as an additive tool in taxonomy.

4.3 EPIDERMAL WALL PATTERN

Both species studied had epidermal wall straight with no degree of straightness varying among them.

4.4 STOMATA DISTRIBUTION

Stomata were found to be present on both species on the lower surface of the epidermis (Abaxial). The stomata found were Anomocytic and Bicytic - paracytic in plate 3.5 and plate 3.7 respectively.

4.5 STOMATA ABNORMALITIES

Contiguous stomata or twin stomata was observed in *Hunteria umbellata*.

Solereder (1908) defined contiguous stomata as pairs of stomata lying side touching one another. According to Pant and Kidwai (1967) and Shah and Gopal (1971), they believed that contiguous development and the presence of contiguous stomata in *Hunteria umbellata* shows that the plant is still evolving.

CONCLUSION

In conclusion, this study on the cytomorphology of *Picralima nitida* and *Hunteria umbellat*a in Edo State reveals distinct stomata types, confirms a chromosome count of 2n=22 for Picralima nitida, and highlights ongoing evolutionary changes in *Hunteria umbellata*. The findings contribute valuable insights into taxonomic relationships and evolutionary dynamics within the Apocynaceae family.

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